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REVIEW

Echinococcosis: Advances in the 21st Century

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SUMMARY Echinococcosis is a zoonosis caused by cestodes of the genus *Echinococcus* (family Taeniidae). This serious and near-cosmopolitan disease continues to be a significant public health issue, with western China being the area of highest endemicity for both the cystic (CE) and alveolar (AE) forms of echinococcosis. Considerable advances have been made in the 21st century on the genetics, genomics, and molecular epidemiology of the causative parasites, on diagnostic tools, and on treatment techniques and control strategies, including the development and deployment of vaccines. In terms of surgery, new procedures have superseded traditional techniques, and total cystectomy in CE, *ex vivo* resection with autotransplantation in AE, and percutaneous and perendoscopic procedures in both diseases have im-

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proved treatment efficacy and the quality of life of patients. In this review, we summarize recent progress on the biology, epidemiology, diagnosis, management, control, and prevention of CE and AE. Currently there is no alternative drug to albendazole to treat echinococcosis, and new compounds are required urgently. Recently acquired genomic and proteomic information can provide a platform for improving diagnosis and for finding new drug and vaccine targets, with direct impact in the future on the control of echinococcosis, which continues to be a global challenge.

KEYWORDS alveolar echinococcosis, cystic echinococcosis, echinococcosis, *Echinococcus*, *Echinococcus granulosus*, *Echinococcus multilocularis*, genetic epidemiology, genome, transcriptome, strains/genotypes, zoonosis

INTRODUCTION

Echinococcosis refers principally to two severe zoonotic tapeworm diseases, cystic echinococcosis (CE) and alveolar echinococcosis (AE), caused by *Echinococcus granulosus sensu lato* and *Echinococcus multilocularis*, respectively (1). CE is cosmopolitan and more common, although a few island countries have declared elimination (2, 3). In areas of endemicity, the annual CE incidence ranges from <1 to 200 per 100,000, whereas that of AE ranges from 0.03 to 1.2 per 100,000 (4). Mortality in untreated or inadequately treated AE patients is >90% within 10 to 15 years of diagnosis (1). The CE mortality rate (2% to 4%) is lower but may increase considerably if inadequate care management is provided. Current estimates of the global burden average 285,500 disability-adjusted life years (DALYs) for human CE (5–7) (>1 million if underreporting is taken into account) and 666,434 DALYs for AE (7). The World Health Organization (WHO) has listed echinococcosis as one of the 17 neglected diseases targeted for control or elimination by 2050 (http://whqlibdoc.who.int/hq/2012/WHO_HTM_NTD_2012.1_eng.pdf). Indeed, major recent advances are set to revolutionize the care management and control of echinococcosis. Nevertheless, improved diagnosis and identification of new drug and vaccine targets are urgently required given the limitations of current diagnostic procedures, the toxicity and poor efficacy of available drugs, the often-inadequate surgical strategy, and the challenges in control and prevention.

In this review we outline the biology and life cycle characteristics of the *Echinococcus* spp. and consider the epidemiology, transmission, and clinical features of echinococcosis. We discuss recent advances in the diagnosis, treatment, care management, prevention, and control of CE and AE and show how genome and transcriptome studies are unravelling details of the developmental biology of *Echinococcus* spp. and their interactions with mammalian hosts, providing important information that can lead to the development of novel interventions and therapies against echinococcosis.

BIOLOGY AND LIFE CYCLE CHARACTERISTICS

The life cycles of the *Echinococcus* spp. are dependent on predator-prey associations involving two mammalian hosts (Fig. 1). Carnivores (canids and felids) serve as definitive hosts for the adult tapeworms, and their herbivorous prey (ungulates, rodents, and lagomorphs) act as intermediate hosts for the metacestodes; humans are generally not directly involved in the transmission of CE or AE, although under certain unique and unusual circumstances, such as reported in the Turkana region of Kenya, humans can act as intermediate hosts for *E. granulosus* (1). The developmental stages of the *Echinococcus* spp., exemplified by *E. granulosus sensu lato*, are shown in Fig. 2 (8, 9). Hundreds to thousands of 3- to 7-mm-long *Echinococcus* sp. adult worms develop in the intestines of their definitive hosts; the last segment (or proglottid) of each worm matures to produce eggs that are released in the carnivore's feces into the external environment. In turn, humans or the intermediate hosts ingest the eggs, which hatch in the intestine to release oncospheres that pass through the portal and lymphatic vessels and reach the liver, where they usually settle and develop as larvae (metacestodes or hydatid cysts); less frequently they may also reach the lungs, brain, bones, or any other organ of the human or intermediate host. Protoscoleces, the fertile forms of

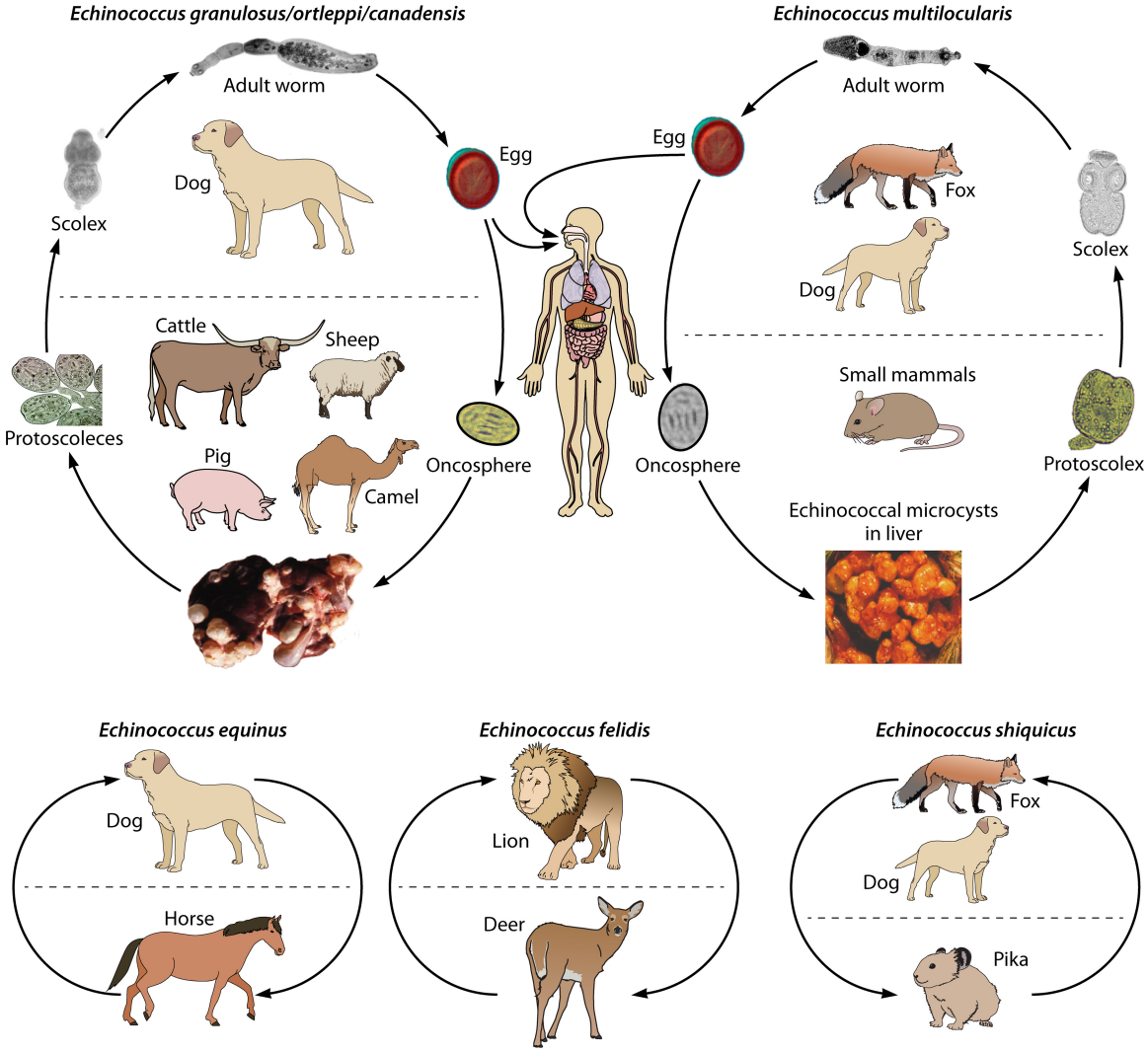

FIG 1 Life cycles of *Echinococcus* spp. Species responsible for human infection (*E. granulosus sensu stricto*, *E. ortleppi*, and *E. canadensis* [belonging to *E. granulosus sensu lato*] and *E. multilocularis*) are shown at the top. Species at the bottom (*E. shiquicus*, a species close to *E. multilocularis*, and *E. equinus* and *E. felidis*, belonging to *E. granulosus sensu lato*) are not known to cause disease in humans. Only the most common definitive and intermediate hosts which play a major role in life cycle/transmission are shown; other hosts may be encountered (especially wildlife hosts for *E. granulosus sensu lato* and domestic hosts for *E. multilocularis*). *E. vogeli* and *E. oligarthra*, which are responsible for polycystic echinococcosis in humans in Central and South America, are not represented in the figure.

the parasite, produced asexually by the metacestode, are released into the hydatid fluid; when ingested by the definitive host, protoscoleces evaginate their scoleces, aided by bile salts, and, after attaching to the intestinal wall, they develop into mature, egg-producing adult worms.

EPIDEMIOLOGY AND TRANSMISSION

Distribution of CE and AE

The pattern of distribution for CE has remained essentially unchanged over the past 2 decades, with areas of high endemicity, including western China, Central Asia, South America, Mediterranean countries and eastern Africa (Fig. 3), and the main risk factors being contact with dogs and raising livestock (3, 10, 11). However, studies in Africa have revealed a significant number of human cases and active transmission in animals, including wildlife, in countries hitherto considered not to be areas of endemicity (12, 13). Five thousand new CE cases are still diagnosed annually in Argentina, Brazil, Chile, Peru, and Uruguay (14, 15). Thirty years of dosing dogs with the anthelmintic drug praziquantel 8 times annually has significantly decreased transmission to humans, but

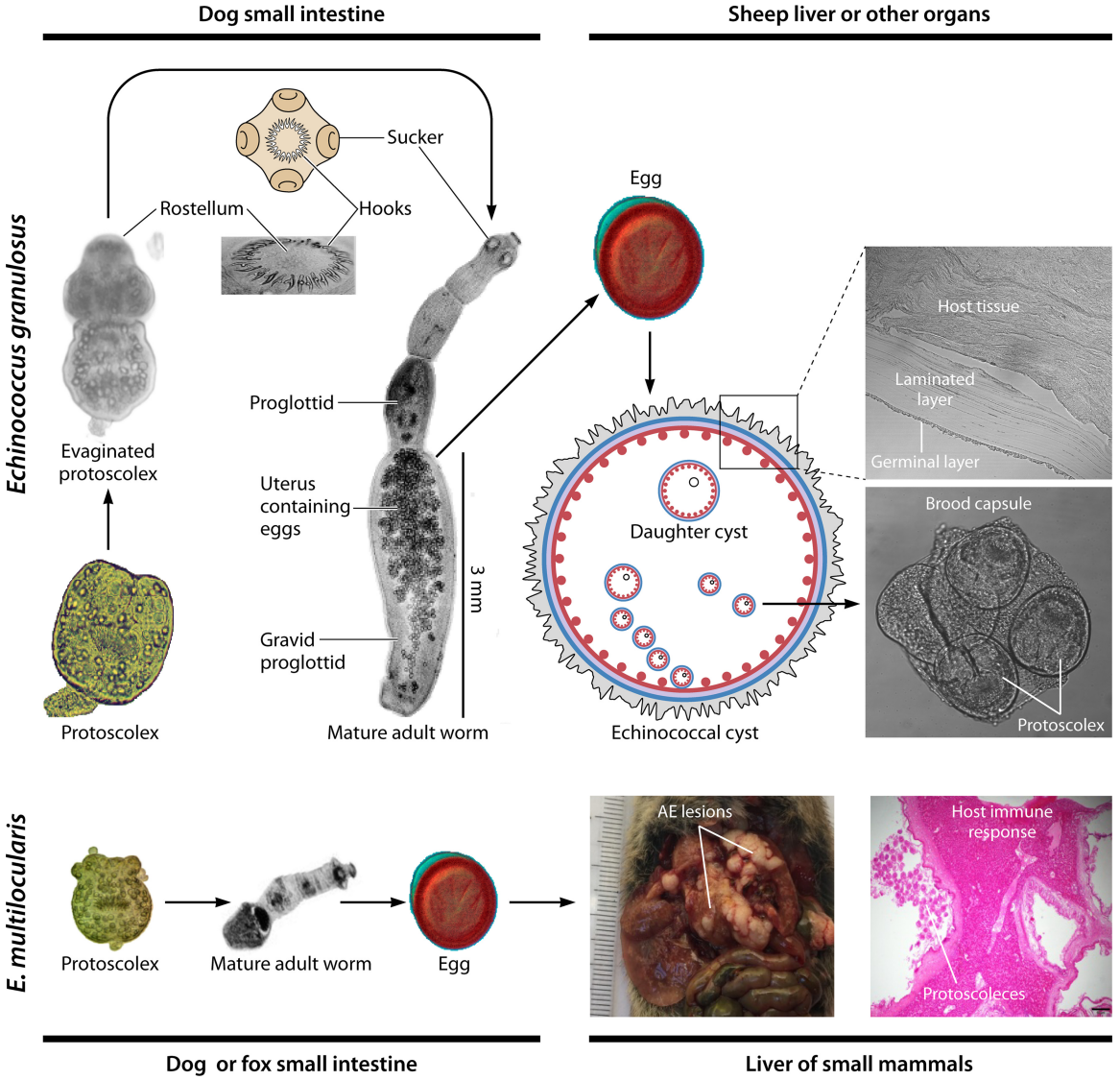

FIG 2 Different developmental stages in *Echinococcus granulosus* and *E. multilocularis*. Growth of the larval cyst is unlimited, and it can, for *E. granulosus*, grow to 30 cm or more in humans, while the adult worm, egg, and protoscolex are limited in size and shape. *Echinococcus* sp. tapeworms have no gut, circulatory, or respiratory organs and have a highly adapted relationship with their mammalian hosts which they exploit for nutrients, signaling pathways, and neuroendocrine hormones. Strobilization is a notable feature of cestode biology, whereby proglottids (segments) bud distally from the anterior scolex, resulting in the production of tandem reproductive units (proglottids) exhibiting increasing degrees of development. *Echinococcus* is monoecious, and the last segment (gravid proglottid) produces diploid eggs that give rise to ovoid embryos, the oncospheres. However, a striking feature of the biology of *Echinococcus* is that the protoscolex has the potential to develop in either of two directions: it may develop into an adult tapeworm producing sexually produced eggs in the dog gut, or, if a hydatid cyst ruptures within the intermediate or human host, each released protoscolex is capable of differentiating asexually into a new cyst, a process termed "secondary" echinococcosis. While a unilocular fluid-filled bladder (cyst) is a feature of *E. granulosus sensu lato* in its larval stage, the metacestode of *E. multilocularis* consists of a mass of small, multilocular vesicles embedded in the immune reaction of the host (granuloma and fibrosis). These multiple and aggregated vesicles grow by proliferation of cells in the germinal layer of the metacestode.

CE is still present in a number of areas in South America (14, 16). CE has been declared eliminated from New Zealand, and Tasmania in Australia is considered to be provisionally free of the disease (17); nevertheless, *E. granulosus* is present on the Australian mainland and is still found in Tasmanian wild and rural dogs, but at low prevalence (18). In Western Europe and North America, most human cases are imported, although an autochthonous cycle of various genotypes within the species group *E. granulosus sensu lato* (see below) is present. However, the lack of accurate case recording currently prevents any precise mapping of the true epidemiological picture; a European Registry of CE (the Heracles project) has been launched to improve this situation (19).

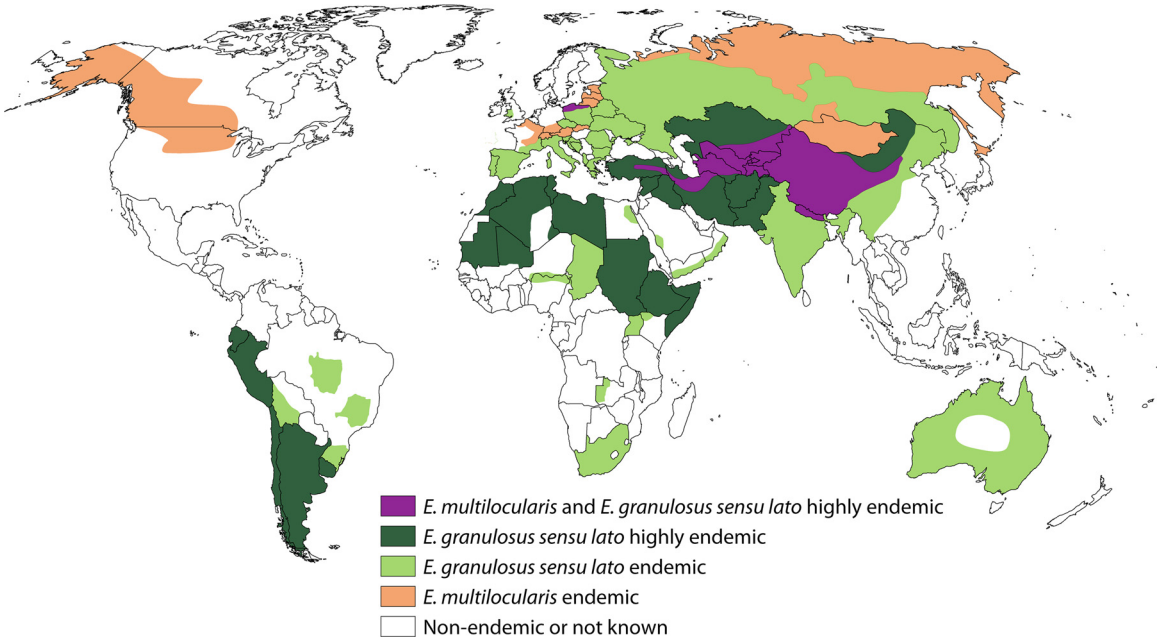

FIG 3 Global distribution *of Echinococcus granulosus sensu lato*, responsible for cystic echinococcosis (CE), and *Echinococcus multilocularis*, responsible for alveolar echinococcosis (AE). The map is based on recent epidemiological studies (1, 13, 19, 247) as far as the current situation has been studied in a given area. The different colors represent a proxy for human prevalence and infection in animal hosts in a given area (to take autochthonous human cases into account only). For AE, the represented disease density is based mainly on the presence of autochthonous AE cases in humans, *E. multilocularis* metacestodes in small mammals, and *E. multilocularis* adult worms in foxes and dogs. For CE, the represented disease density is based mainly on the presence of autochthonous human cases of CE and of *E. granulosus sensu lato* metacestodes (irrespective of species or genotype) in intermediate hosts, including sheep, cattle, equids, and camels. For more accurate and detailed data and maps, see a recent comprehensive review paper by Deplazes et al. (13).

AE has been a public health concern in northern Japan for the past 40 years (20–22). Mass screening with ultrasound (US) and serology in China have confirmed a high incidence of AE on the Tibetan plateau (in Qinghai, Sichuan, and Tibet [23]) and show that AE prevalence, especially in children, is higher than that of CE in several areas (24). Among the 18,235 estimated new AE cases per year globally, 91% occur in China (7), with human prevalence of >3% in some areas (25). AE is also endemic in Central Asia, with high endemicity of both *E. multilocularis* and *E. granulosus* in Kazakhstan and Kyrgyzstan (26–29). In Europe, the prevalence of *E. multilocularis* in definitive and intermediate hosts increased markedly within the first 15 years of this century, and the geographic distribution of fox infections is far broader than earlier reported; urban foxes may be involved in transmission (13, 17, 30–33). Human cases have been found in European countries previously considered to be free of AE, and the situation in the Baltic region has become worrisome; in addition, AE incidence has doubled in the previously recognized areas of endemicity of France, Switzerland, Germany, and Austria (13, 34–36).

In regard to North America, the north-central United States, northwestern Alaska, and northwestern Canada have long been areas of *E. multilocularis* endemicity, but the parasite's geographic range appears to be expanding due, at least in part, to increased and improved sampling efforts and the targeting of definitive hosts other than foxes (such as coyotes [*Canis latrans*]) (13). AE had not been considered a mainstream human health issue in North America other than in Alaska until recently, and *E. multilocularis* has not been reported from Mexico or the southern United States (13). However, human cases were reported in Alberta, Canada, in the past decade (37) as well as in Quebec and Manitoba (unpublished reports to a WHO Collaborating Centre); molecular analysis of *E. multilocularis* in Alberta suggests that coyotes are important definitive hosts and that a European strain is involved, perhaps through carnivores imported from Europe, and not the local endemic "Alaskan" strains (38, 39).

TABLE 1 Current recognized species within the genus *Echinococcus* and their preferential hosts and geographic distribution

Species	Definitive host(s)	Intermediate host(s)	Human cases	Distribution
Echinococcus granulosus sensu stricto	Domestic dog, wolf, dingo, jackal, other canids	Sheep, goat, cattle, pig, camel, buffalo, horse, wild ungulates, marsupials, etc.	Yes	Cosmopolitan
Echinococcus canadensis	Domestic dog, wolf	Pig, camel, cervids	Yes	Eurasia, Africa, North and South America
Echinococcus ortleppi	Domestic dog	Cattle	Yes	Eurasia, Africa
Echinococcus felidis	Lion	Hyena, warthog, zebra, wildebeest, bush pig, buffalo, various antelopes, giraffe, hippopotamus	Not reported	Africa
Echinococcus equinus	Domestic dog	Horse, other equids, cervids	Not reported	Eurasia, Africa
Echinococcus multilocularis	All fox species, wolf, raccoon dog, domestic dog, cat	Arvicoline and microtine rodents and small herbivorous mammals, including lagomorphs (e.g., pika); pigs, boars, horses, cattle, nutrias, nonhuman primates, and dogs are accidental hosts	Yes	Eurasia, North America
Echinococcus oligarthra	Wild felids (e.g., *Puma concolor* [puma])	*Dasyprocta azarae* (agouti), *Didelphis marsupialis* (opossum)	Yes	Central and South America
Echinococcus vogeli	Bush dog, domestic dog	*Cuniculus paca* Linnaeus, 1766 (paca)	Yes	Central and South America
Echinococcus shiquicus	Tibetan fox	*Ochotona curzoniae* (Tibetan plateau pika)	Not reported	Tibetan Plateau

The distribution of "neotropical echinococcosis," i.e., echinococcosis due to *Echinococcus vogeli* and *Echinococcus oligarthra* (see comment below on its correct taxonomic spelling), remains limited to South America (40); newly recognized human cases of *E. vogeli* infection in new areas, such as French Guyana in eastern South America (41, 42), are likely the result of improved diagnosis and molecular identification of the disease (43).

Genetics and Genetic Epidemiology

A major change in the epidemiological picture for CE has come about as a result of the redefinition of the *Echinococcus* spp. causing the disease. Until relatively recently, *E. granulosus* was considered a single species, but it is now recognized as having extensive genetic diversity, with distinct strains/genotypes exhibiting differences in pathology and differing responses to drugs and the defined recombinant vaccine EG95 (see "Vaccination of intermediate hosts" below) for ovine CE (3). The application of mitochondrial DNA sequencing has resulted in the recognition of 10 genotypes (G1 to G10) and their accurate identification in molecular epidemiological surveys of CE in different geographical settings and host assemblages (44). Accordingly, the 10 strains/genotypes of *E. granulosus sensu lato* have been demarcated into 5 species, including *E. granulosus sensu stricto* (the former "sheep strain," G1 to G3), *Echinococcus equinus* (horse strain, G4), *Echinococcus ortleppi* (cattle strain, G5), *Echinococcus canadensis* (camel strain, G6; pig strain, G7; G9, probably a variant of the pig strain; and cervid strains, G8 and G10), and *Echinococcus felidis* ("lion strain") (12, 45–48). Currently, 9 species, listed in Table 1, are recognized in the genus *Echinococcus* (49); the life cycles of some of these are shown in Fig. 1. *E. granulosus sensu stricto* is the most widely distributed (Fig. 3), with other species being focal.

The use of mitochondrial DNA and/or DNA microsatellites, such as the EmsB marker (50), has made discrimination between distinct genotypes of *E. multilocularis* possible. The influence of these genetic differences on increased prevalence or severity of AE in humans is unknown, but such genetic analysis is useful for tracking the transmission of a particular genotype from one area to another (51–54). The Alaskan origin of *E. multilocularis* found in the Norwegian Svalbard islands and the European origin of *E. multilocularis* found in Alberta, Canada, are examples where molecular markers have proved useful (37, 55). *Echinococcus shiquicus*, which is transmitted between Tibetan foxes/dogs (56) and the plateau pika (*Ochotona curzoniae*), is a new species found only in the Tibetan region, with no human cases thus far recorded (56–59). The less common *E. vogeli* and *E. oligarthra* (replacing the previously used incorrect taxonomic spelling "*E.*

oligarthrus" ["arthra" being the plural of the Greek noun "arthron," which means "joints" {i.e., proglottids}, and not an adjective subject to gender agreement with "*Echinococcus*" {48}]) are restricted to Central and South America (42, 60, 61). Analysis of nuclear and mitochondrial markers revealed that populations of *E. vogeli* (Brazilian Amazon) and *E. oligarthra* (Argentina) are genotypically variant (60, 62).

CLINICAL FEATURES

The clinical features of echinococcosis have been comprehensively described (1, 63). In CE these are associated with damage or dysfunction of target organs, particularly the liver (70%) and lungs (20%), with the remainder including the brain, spleen, kidney, and heart. Almost all primary AE lesions are in the liver. Clinically, most AE and CE patients present late at clinics or hospitals. Population screening has shown that CE liver cysts in humans grow very slowly, with more than half of cysts showing no change in size in 10 years and one-third growing less than 3 cm; mean cyst growth in cases with a prolonged follow-up was 0.7 cm (8). The early stages of CE and AE do not cause symptoms, and CE cysts and AE lesions can remain asymptomatic for 10 to 15 years; consequently, children comprise only a small percentage of echinococcosis patients. Clinical symptoms usually appear when a cyst reaches more than 10 cm in diameter in the liver or when more than 70% of the organ volume is occupied by a cyst or cysts, resulting in physical compression or damage to bile ducts, hepatic veins, the portal vein, or the hepatic artery. Various symptoms may be due to compression or damage to bronchia in the lungs or various structures of the brain, which can also result in life-threatening complications. In any organ, compression of vital structures may be symptomatic even with small or medium-sized cysts.

Symptomatic CE patients with liver cysts most often present with upper abdominal discomfort and poor appetite; compression of bile ducts may lead to jaundice. On palpation, a tumor-like mass, hepatomegaly, or abdominal distension may be found. Chest pain, cough, or hemoptysis can be indicative of cysts in the lung, and cyst rupture into the bronchi may result in the expulsion of hydatid materials. Any neurological symptom (signs of intracranial hypertension, epilepsy, all types of paralysis, etc.) may be observed in patients with brain cysts. In any organ, cyst rupture can induce fever, urticaria, eosinophilia, and anaphylactic shock. Potentially lethal allergic reactions due to cyst rupture and even minor fissures have long contraindicated any puncture of a CE cyst. Antigen leakage associated with such fissures may reveal the usual development of specific IgE antibodies, common for *E. granulosus sensu lato* and *E. multilocularis*, which is part of the predominant Th2-type immune response in echinococcosis, with high levels of interleukin-5 (IL-5) in both AE and CE (64). Despite the presence of IgE antibodies and demonstrated possible basophil activation (65), eosinophilia and allergic reactions are very uncommon in AE because of the different structure of the parasitic lesions, with dense fibrosis preventing vesicle fluid leakage; these reactions may be observed in rare cases of blood dissemination of lesion fragments (64). Routine imaging or population mass ultrasound (US) screening of the liver may identify asymptomatic cysts (66), and the procedure is extremely important for finding early-stage AE patients in areas of endemicity (23).

Faster growth of cysts in CE patients with AIDS suggests that immune suppression may play a role in CE progression (67, 68). Conversely, the concept of an enhancing effect of CE on the occurrence of cancer in the population, because of a defect in immune surveillance linked to an *Echinococcus*-induced tolerance state, has been raised recently (69, 70), although this hypothesis has not been rigorously evaluated. In areas where CE is endemic, the simultaneous occurrence of two frequent diseases cannot be ruled out, and preliminary assessment from hospital medical information systems of the occurrence of cancer in 2,350 patients with CE compared with patients without CE showed no difference (Bo Ran, First Affiliated Hospital of Xinjiang Medical University, personal communication). However, the promoting effect of cancer or its treatment on parasite growth seems to interfere far less with the occurrence and/or disease course in CE than in AE (71).

Genetic variation of the human leukocyte antigen (HLA) system is associated with the occurrence and/or progression of AE lesions in humans (72, 73); patients with the HLA-DR3 DQ2 haplotype were shown to have more severe disease and a more pronounced Th2-type immune response, associated with a deeper tolerance status (74, 75). The effects of immune suppression on *E. multilocularis* growth are well known in animal models (76). They were first reported in human cases after liver transplantation performed to treat AE and in patients with AIDS. Early recurrence of AE was observed in transplanted patients and was found associated with the level of immune suppression resulting from the treatment used to prevent liver rejection (77); unusually rapid progression of lesions and the corrective effect of antiretroviral therapy were observed in AIDS patients, including children (67, 78).

After AE occurrence was reported in transplant patients, other than those undergoing liver transplantation for AE, and in patients treated for malignant or chronic inflammatory diseases (79–81), a systematic study based on the French National Registry of AE (FrancEchino) confirmed the significant and recent increase (from the beginning of the 21st century) in the occurrence of AE in such patients (71). Acquired therapeutic immunosuppression (which combined chemotherapy, corticosteroids, and biotherapy such as anti-tumor necrosis factor [anti-TNF] agents) appears to be the main factor for AE occurrence and its fast progression. Unusual presenting symptoms, such as bacterial abscess-like acute clinical symptoms, and misleading imaging findings, such as abscess-like, metastasis-like, or hemangioma-like aspects on computed tomography (CT) scans, contributed to delayed diagnosis. This resulted in incorrect therapeutic management of a number of these patients, such as those undergoing cancer treatment intensification, which further enhanced metacestode growth, or ineffectual radiofrequency ablation attempts on the presumed liver "tumor" or "metastasis" (71). Negative serology, likely due to the patient's immune suppression status, is also an issue and makes pathological and/or molecular identification of the metacestode often necessary before diagnosis can be confirmed (71). Whether the patients were actually infected with *E. multilocularis* eggs during the period of therapeutic immune suppression or the symptoms were due to a reactivated, dormant metacestode resulting from a previous infection remains to be established (71).

Diagnosis

General comments. Imaging techniques are essential for diagnosis, with the relatively inexpensive and portable ultrasound (US) widely used to diagnose CE or AE liver lesions; X-ray is used for lung cysts. Both techniques are used for diagnosis and population screening and for follow-up (82–84). Serology, i.e., detection of specific antibodies against *Echinococcus* sp. antigens, is a confirmatory step with various levels of sensitivity/specificity correlating with the involved species, lesion location, or antigen used (63) (Fig. 4). Mass population screening of CE and AE in areas of endemicity using US is considered the best method for early diagnosis. In addition to organized mass screening, routine health checks and systematic follow-up of associated diseases, including US examination, are now a major approach in echinococcosis diagnosis. This has contributed to the diagnosis of asymptomatic cases in the general population and to changes in the presentation of AE cases, especially in Europe (85). Systematic follow-up of patients with malignant diseases and a variety of chronic diseases, by using US, CT scan, and fluorodeoxyglucose-positron emission tomography (FDG-PET), may also have contributed to the early diagnosis of AE in such individuals (71).

Imaging and classification of CE and AE lesions. Based on US imaging, the World Health Organization Informal Working Group on Echinococcosis (WHO-IWGE) has classified hepatic CE cysts into five types, CE1 to CE5, and AE lesions into different PNM (parasite lesion, neighbor organs, metastases) types (1, 86–88), which provides basic information for clinicians to make treatment decisions (Fig. 5; Table 2). Harmonization of disease assessment at the international level is crucial to progress toward an evidence-based and stage-specific strategy for treatment (89). New classification of US images and the associated classification of computed tomography (CT) images in AE

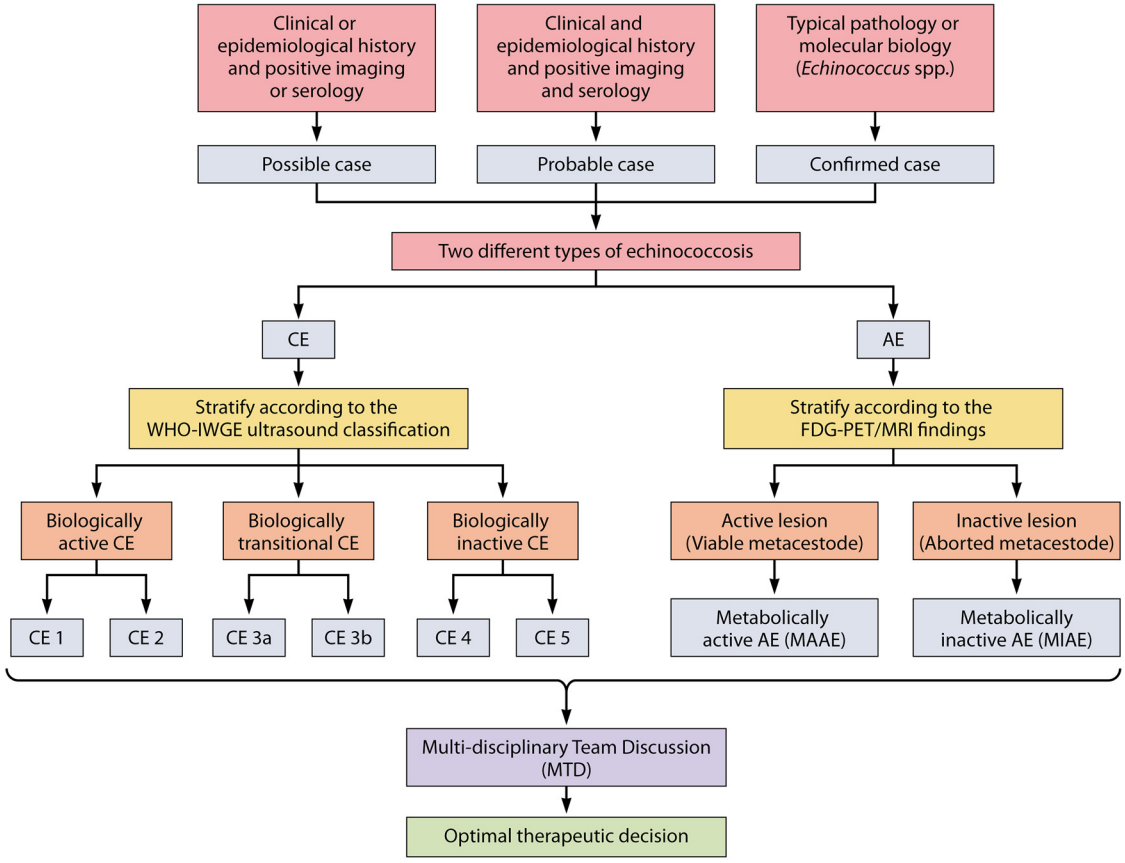

FIG 4 Algorithm for the diagnosis of cystic echinococcosis (CE) and alveolar echinococcosis (AE). Definitions of "possible," "probable," and "confirmed" cases refer to the "Expert consensus for diagnosis and treatment of echinococcosis in humans" (86). CE1 to CE5 refer to the "WHO-IWGE [World Health Organization Informal Working Group on Echinococcosis] international classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings" (86) and Fig. 5. FDG-PET, fluorodeoxyglucose-positron emission tomography (increased uptake of FDG by the periparasitic immune response is the currently accepted evidence for AE lesion metabolic activity) (94). MRI, magnetic resonance imaging (identification of typical microcysts on T2-weighted images at MRI is a surrogate marker for AE lesion metabolic activity) (98).

have been proposed (90, 91) and are currently being tested on patients at European and Chinese centers in order to evaluate their usefulness for diagnosis and follow-up.

The challenge in imaging diagnosis of echinococcosis is detecting small cysts/lesions (<2 cm in diameter). Contrast-enhanced ultrasonography (CEUS) may be used for detecting small AE lesions and differentiating them from abscesses and tumors based on pulsating blood flow imaging (92–94). Fluorodeoxyglucose (FDG) uptake surrounding AE lesions is higher than in other areas, and FDG-positron emission tomography (FDG-PET) has become the favored reference tool to evaluate their metabolic activity (Fig. 4 and 6) (95–98). Color and pulsed doppler US, dual-energy CT or spectral CT, and diffusion-weighted magnetic resonance imaging (MRI) might also be useful in detecting blood supply and the metabolism of lesions (97, 99) but they cannot be recommended without further evaluation (97). In CE, MRI appears to be of better diagnostic value than CT scanning (100), and both procedures are complementary for AE and should be performed to provide sufficient information for therapeutic decision-making (Fig. 4) (97). However, MRI T2-weighted microcystic images are pathognomonic of AE lesions (Fig. 6), and in difficult cases US-guided core-needle biopsy is reliable and effective in combination with DNA diagnostic testing (101) or specific immunostaining (102).

Serology. The sensitivities and specificities of serological tests for CE and AE have been comprehensively reviewed (103). Hydatid fluid (HF) is the major antigenic source for echinococcosis immunodiagnosis, with the HF lipoproteins antigen B (AgB) and

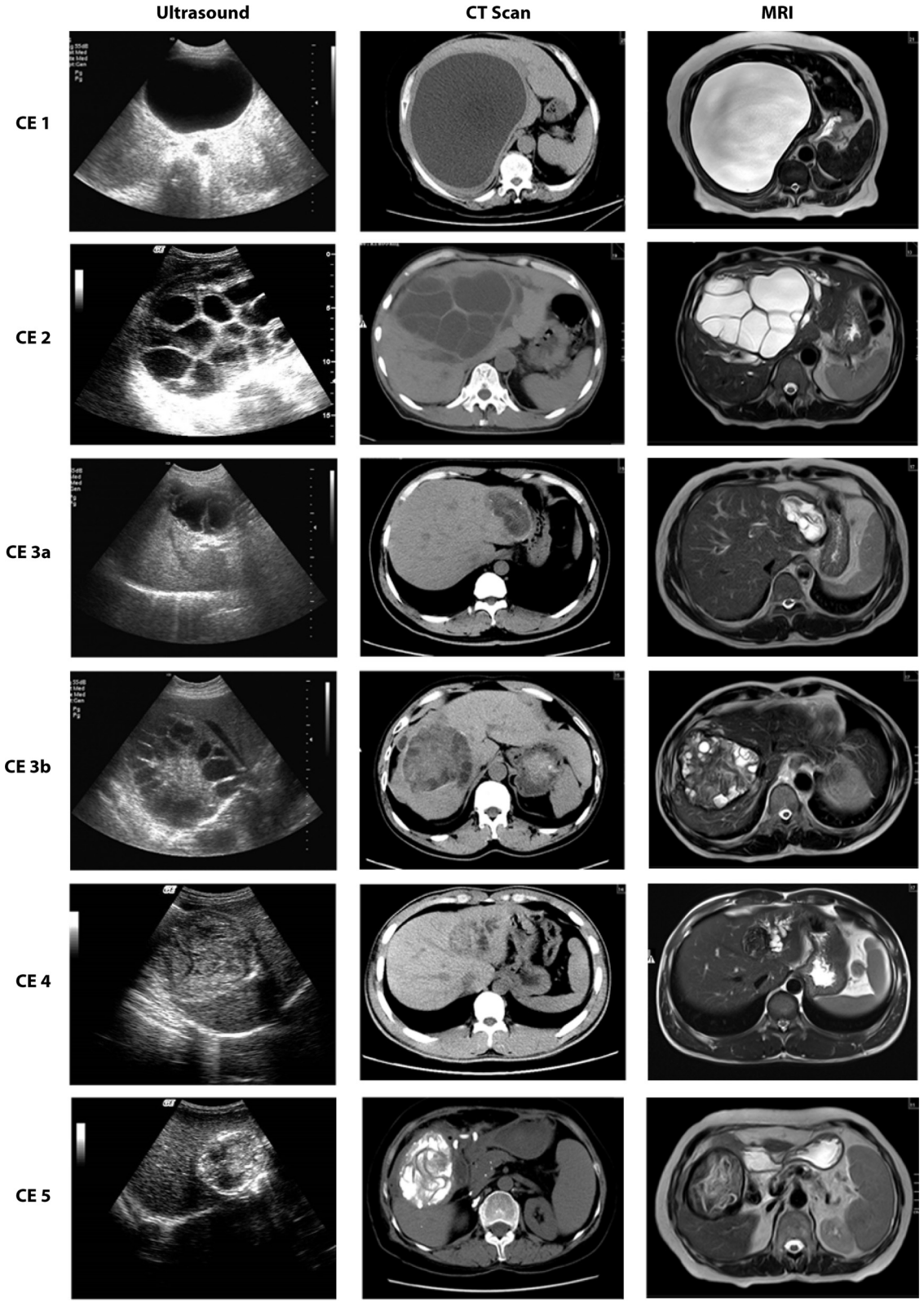

FIG 5 Imaging of cystic echinococcosis. The description of ultrasound images is according to the WHO Informal Working Group on Echinococcosis (WHO-IWGE) international classification (86) and corresponding images were obtained from plain computed tomography (CT) scanning and T2-weighted magnetic resonance imaging (MRI) in representative cases. In the international classification, types CE1 and CE2 correspond to "active stages," types CE3a and b to the "transitional stage," and types CE4 and CE5 to "degenerating stages" (CT and MRI images provided by Liu Wenya, Department of Radiology, First Affiliated Hospital of Xinjiang Medical University, Urumqi, People's Republic of China).

TABLE 2 WHO Informal Working Group on Echinococcosis PNM classification and staging of alveolar echinococcosis[a]

Classification	Description
P	Hepatic localization of the parasite
PX	Primary AE lesion cannot be assessed
P0	No detectable AE lesion in the liver
P1	Peripheral lesion(s) without proximal vascular and/or biliary involvement
P2	Central AE lesion(s) with proximal vascular and/or biliary involvement of one lobe[b]
P3	Central lesion(s) with hilum vascular or biliary involvement of both lobes and/or with involvement of two hepatic veins
P4	Any liver lesion with extension along the vessels[c] and the biliary tree
N	Extrahepatic involvement of neighboring organs (diaphragm, lung, pleura, pericardium, heart, gastric and duodenal wall, adrenal glands, peritoneum, retroperitoneum, parietal wall [muscles, skin, bone], pancreas, regional lymph nodes, liver ligaments, kidney)
NX	Not evaluable
N0	No regional involvement[d]
N1	Regional involvement of contiguous organs or tissues
M	Absence or presence of distant metastases (lung, distant lymph nodes, spleen, central nervous system, orbital, bone, skin, muscle, kidney, distant peritoneum, and retroperitoneum)
MX	Not completely evaluated
M0	No metastasis[e]
M1	Metastasis
PNM stages	
I	P1 N0 M0
II	P2 N0 M0
IIIa	P3 N0 M0
IIIb	P1–P3 N1 M0, P4 N0 M0
IV	P4 N1 M0, any P any N and/or M1

[a]According to reference 88.
[b]For classification, the plane projecting between the bed of the gall bladder and the inferior vena cava divides the liver in two lobes.
[c]Vessels mean inferior vena cava, portal vein, and arteries.
[d]Including a negative chest X ray or thoracic CT result.
[e]Including a negative chest X ray or thoracic CT result as well as a negative brain CT result.

antigen 5 widely used in serological assays for CE (1). Although the Casoni intradermal test exhibits low specificity and sensitivity (104), it may be used for confirming the results of US or other physical imaging methods (Xinyu Peng, personal communication). Poor standardization and ethical issues regarding reagents from animal origin injected into humans have, however, considerably limited the use of skin tests for echinococcosis diagnosis. Reported sensitivities and specificities of serological methods for testing CE patients confirmed by surgical resection vary from 60% to 90%. Use of enriched antigen 5 (105) and recombinant antigens based on repeated tandem *E. granulosus* AgB (2B2t antigen) and recombinant Ag5 (106) increases diagnostic value. A large number of other novel antigens, including the tegumental protein EgTeg (107), alkaline phosphatase (EgAP) (108), and EpC1 (109), exhibited greater than 90% sensitivity and specificity on selected serum samples. However, their performance has never been evaluated on a large scale, and none of the reported antigens are sufficiently sensitive or specific to be used as a first-intent tool for diagnosis or mass population screening (110, 111). One major issue is the lack of appropriate antigens with the required sensitivity for the serological detection of small CE cysts in the liver and cysts of any size in lungs. Encystment of the metacestode, preventing the stimulation of antibody-producing cells and thus causing the absence of measurable levels of antibodies generated against *Echinococcus* sp. antigens, can explain many negative results.

AE serology is more reliable. Em2 and Em492, which represent constituents of the excretory/secretory (ES) fraction of intact metacestodes, as well as EmAP and EmP2, are specific for *E. multilocularis* infection (76). EM10, or its derivatives EmII/3 and Em18, which are encoded by part of the EM10 gene sequence, show high diagnostic performance for confirming AE (112). A commercialized Em2^{plus} enzyme-linked immunosorbent assay (ELISA) (Bordier, Crissier, Switzerland) has been used extensively for clinical diagnosis of AE (113), with sensitivity and specificity exceeding 90% (114). Nevertheless,

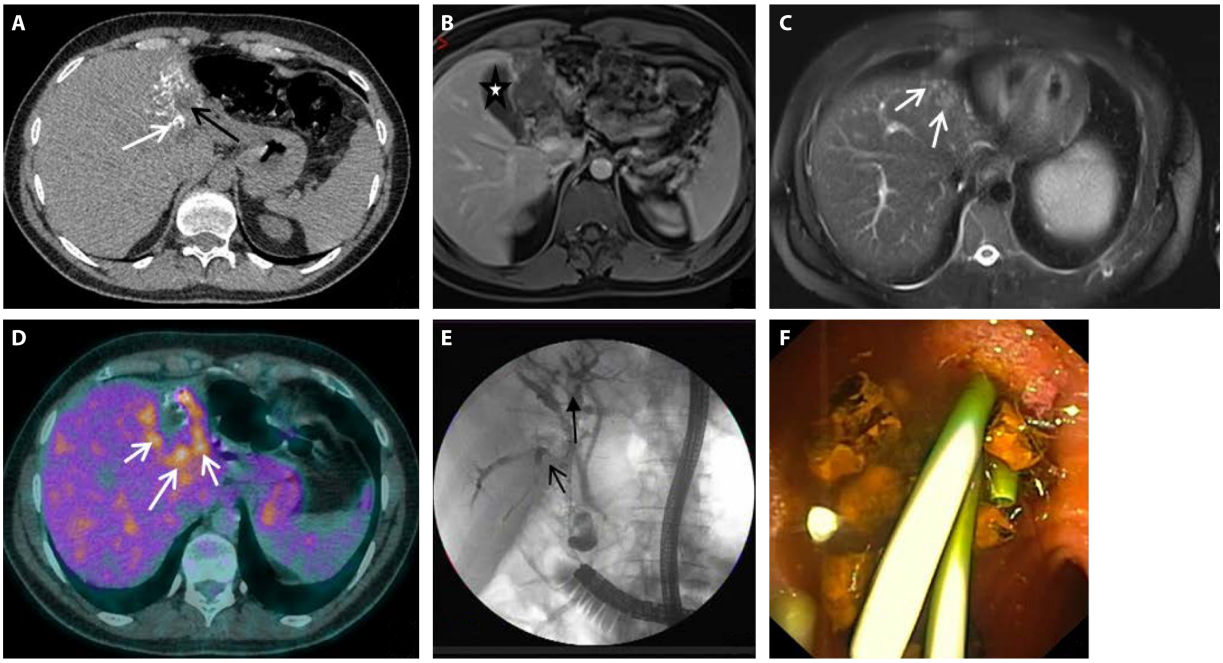

FIG 6 Complementarity of imaging techniques for the diagnosis and preoperative assessment of alveolar echinococcosis (AE) lesions in a patient with portal vein and bile duct invasion by the parasitic lesions. (A) On computed tomography (CT) scanning, the lesion shows the characteristic heterogeneous aspect of AE, with hyperdense calcifications (white arrow) and hypodense area (central necrosis) (black arrow). (B) Fat-suppressed T1-weighted magnetic resonance (MR) image after gadolinium injection at the portal venous phase shows an atrophy of the left liver and invasion of the left portal vein and of the left intrahepatic biliary tree. The lesion is at the contact of the gallbladder (white star) which is not invaded. (C) T2-weighted MR images show the presence of hyperintense microcysts (white arrows), pathognomonic of AE, but also a solid component (Kodama type II). (D) Fluorodeoxyglucose (FDG) uptake in positron emission tomography (PET) is markedly increased at the periphery of the lesion (white arrows). (E) Assessment of biliary tree involvement and treatment by perendoscopic stenting in a patient with late postoperative biliary complications of AE. Endoscopic retrograde cholangiopancreatography (ERCP) was performed (with a colonoscope because of previous gastrectomy), showing dilation of the extrahepatic and intrahepatic biliary tree with several defects (black arrows) because of biliary stones due to chronic biliary obstruction and bacterial superinfection. (F) Perendoscopic stenting via ERCP. After balloon dilation followed by extensive lavage with isotonic saline and stone extraction, 3 plastic stents (size, 7 and 10 French) are inserted in the stenosis of the bile duct (endoscopic view).

serology cannot be used as a first-intent diagnostic tool for mass screening in areas of endemicity, where a proportion of the human population exhibits positive serology without AE lesions (115). Serology has also been shown to be frequently negative in immunosuppressed individuals with AE, and thus, it should not be used on its own as an argument against a diagnosis in such patients (71).

For both CE and AE, serology is thus now used only to confirm imaging results; it may also provide some insight into the infection pressure on a given population (e.g., children) in a particular geographic area. Serology results are included in the definition of "possible" and "probable" cases by the Expert Consensus of the WHO-IWGE (86). The uses of imaging, serology, and molecular identification of the metacestode in the diagnostic strategy are shown in the algorithm proposed in Fig. 4.

Protein biomarkers. *Echinococcus* spp. can survive in humans for a long time through active regulation of the host immune response by the secretion of proteins at the interface of parasite and host tissues. Profiling HF protein composition and excretory/secretory (ES) products provides valuable information on parasite survival strategies and the molecular mechanisms of parasite-host interaction. In addition, analysis of the protein profiles can help in identifying potential molecular markers for developing diagnostic and follow-up tools. Proteomic analysis of the composition of CE (116, 117) and AE (118) cyst/vesicular fluids has identified hundreds of proteins from both *Echinococcus* spp. and the host that may help differentiate subpopulations of patients. Characterization of ES proteins from *E. granulosus* adult worms (119) and protoscoleces (120, 121) and *E. multilocularis* protoscoleces (122) also shows promise for identification of potential diagnostic markers.

Application of proteomics to patient care management is in its infancy. Specific

immunodominant epitopes of *E. granulosus* HF change as the disease progresses (e.g., from CE1 to CE2) (123), and the HF protein composition is different in different organ locations of the cysts (124); this could explain the well-known differences in the host antibody response correlating with the stage and location of cysts. Immunoreactive proteins from *E. multilocularis* vesicular fluid have been recently identified and quantified, and comparative proteomics revealed 9 proteins (actin modulator protein, fucosidase alpha L1 tissue, prosaposin a preprotein, glutathione *S*-transferase, beta-galactosidase, NiemannPick C2 protein, elongation factor 2, cathepsin b, and H17g protein tegumental antigen) that were more abundant in immunoprecipitation eluates from albendazole (ABZ)-nonresponders than in those from ABZ-responder AE patients, suggesting that detection of antibodies against these proteins by ELISA could be helpful in monitoring the course of AE under ABZ treatment (118).

DNA detection. Recently developed DNA-based methods, such as quantitative and/or nested PCR assays, are highly sensitive, reasonably specific, and able to distinguish *Echinococcus* species from each other and from other cestodes; they can, as discussed above, discriminate the various genotypes of *E. granulosus*, including following clinical biopsy of a suspected CE or AE case, and identify infected mammalian host species (125–130). Other examples include the identification of *E. vogeli* in patients infected in an area not previously considered to be an area of endemicity (42), in revealing the reemergence of *E. ortleppi* in France (131), in the retrospective identification of an *E. multilocularis* strain in a historical human case of AE in the United States (132), and in the detection of *E. multilocularis* infection in primates (133). DNA identification methods are now routinely used on biopsy or fine-needle cytology specimens for the diagnosis of AE in patients with unusual imaging aspects and/or with negative serology, typified by immunosuppressed patients (71), and they form part of the definition of "confirmed cases" of the WHO-IWGE Expert Consensus (86) (Fig. 4). As is discussed further below, molecular diagnosis, including loop-mediated isothermal amplification (LAMP), can be used as a first-line screen for *Echinococcus* spp. in the field (134–137) and to detect *Echinococcus* sp. egg DNA in environmental samples as an important step for identifying high-risk contaminated areas and for defining the actual routes of human infection (138–142).

CARE MANAGEMENT

Based on image classification and following a stage-specific approach, various options are possible, alone or combined, for the treatment of both CE and AE, including (i) surgery, (ii) nonsurgical interventions, (iii) anti-infective benzimidazole drug treatment, and (iv) a "watch-and-wait" approach (Fig. 7). The current recommendations for echinococcosis management take the cancer-like nature of the disease, which is prone to recurrence, into account and thus use the model of cancer care management which promotes a multidisciplinary approach, with interdisciplinary team consultations for therapeutic decisions, the combination of surgical and drug treatments, a long-term follow-up of patients, the establishment of international recommendations, and the creation of reference centers (86).

Treatment of CE

With CE, treatment centers on cyst type according to the WHO-IWGE US classification (1) (Fig. 5), size, location, and presence/absence of complications, as well as available medical expertise and equipment (86). Curative treatment is achieved by the complete removal of the cyst, regardless of location. If the cyst with all its layers (including adventitia) cannot be removed totally, which is the case with sub-total cystectomy and all types of partial cystectomy and with the percutaneous "PAIR" (puncture, aspiration, injection, and reaspiration) technique, the therapeutic procedure should be complemented with the use of protoscolecidal agents. Intraoperative dissemination of protoscolex-rich fluid during surgery and insufficient killing of protoscoleces and germinal membrane during the percutaneous procedures are major causes of

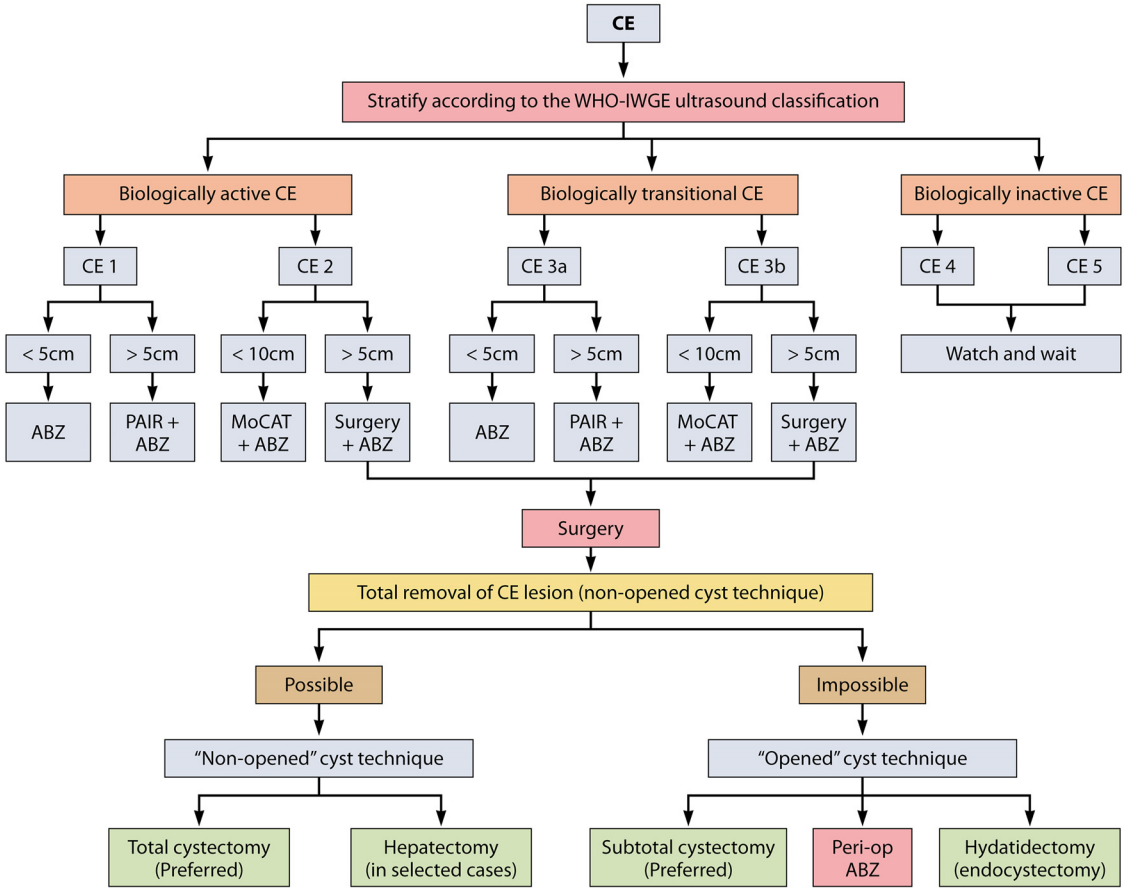

FIG 7 Algorithm for the treatment of cystic echinococcosis (CE), based on "WHO-IWGE international classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings" (86) and Fig. 4 and 5. WHO-IWGE, World Health Organization Informal Working Group on Echinococcosis; ABZ, albendazole; PAIR, puncture-aspiration-injection-reaspiration (nonsurgical percutaneous interventional technique for treatment of CE cysts).

CE recurrence (86). An algorithm that describes the recommended therapeutic strategy for CE is given in Fig. 7.

Use of protoscolecides during CE surgery. The intraoperative dissemination of protoscolex-rich hydatid fluid during surgery or the PAIR procedure for CE is a major cause of cyst recurrence (86). Injection of a protoscolecide into CE cysts to reduce the risk of spillage of viable protoscoleces and possible recurrence is an integral part of the surgical technique employed by many surgeons around the world (143). A very broad spectrum of protoscolecidal agents, from warm water (144) to the highly toxic formalin (145), have been tested over the past 50 years, but the feasibility, safety, and efficacy of many of these compounds have generally not been determined. Details of the concentrations and modes of administration of currently available and tested protoscolecides are provided in Table 3. Serious complications have limited the use of some of these; formalin and betadine should never be used under any circumstances. However, the majority, albeit less harmful than these two compounds, resulted in serious biliary, gas embolism, renal, and toxic complications that limited their use (146) (Table 3); toxicity to bile duct mucosa explains why communication between the CE cyst and the bile ducts must be carefully checked before the use of any protoscolecidal agent. There have been considerable efforts made to discover additional potential protoscolecides, including plant extracts and the use of physical methods *in vitro*, but details of their clinical application are lacking. Currently, the WHO-IWGE recommends 20% hypertonic saline as the preferred protoscolecide in surgery and 20% hypertonic saline or 95% alcohol in PAIR (86), but rigorous, high-quality comparative studies on these protoscolecidal agents are still awaited.

TABLE 3 Protoscolecides tested for use in CE surgery or PAIR[a]

Category	Tested agents (references)	Use:			Limitations	Recommendation	Cautions
		In surgery	In PAIR	*In vitro*			
Chemical compounds	Albendazole (147), alcohol (148), betadine (149), cetrimide-chlorhexidine solution (150), chenodeoxycholic acid (151), cyclosporin A (152), formalin (145), FBG (153), hydrogen peroxide(154), octenidine dihydrochloride (155), pyridinyl imidazole derivative (156), silver nitrate (0.5%) (157), synthesized silver nanoparticles (158), praziquantel (159), taurolidine (160), thymol (161)	Alcohol, betadine, cetrimide-chlorhexidine solution, formaldehyde solution, hydrogen peroxide, silver nitrate (0.5%)	Albendazole, alcohol, betadine, hypertonic saline, sliver nitrate	Chenodeoxycholic acid, cyclosporin A, FBG, pyridinyl imidazole derivative, synthesized silver nanoparticles, praziquantel, taurolidine, thymol	The efficacy of these agents is concentration dependent and thus demonstrated toxic effects on bile duct mucosa, leading to sclerosing cholangitis	For surgery (20% hypertonic saline), careful monitoring of patients' electrolytes and appropriate protection (with isotonic 0.9% saline-moistened laparotomy towels) to prevent hyperosmolar damage to the peritoneum are recommended; for PAIR, (hypertonic saline and >95% alcohol), communication of the cyst with bile ducts must be checked (and repaired, if any found, at surgery) before the use of any protoscolecide	Formaldehyde solution and betadine must not be used; gas embolism has been reported with the use of hydrogen peroxide. and this protoscolecide should not be used in deep highly vascularized cavities
Natural extracts	*Allium sativum* extract (162), *Berberis vulgaris* aqueous extract (163), *Foeniculum vulgare* mill extract (164), *Myrtus communis* oil extract(165), *Nigella sativa* oil (166), *Pistacia vera* oil extract (167), *P. khinjuk* methanolic extract (168), *P. atlantica* hydroalcoholic extracts (169), *Peganum* seed extracts (170), *Salvadora persica* root extracts (171), *Zataria multiflora* methanolic extract (172)	No reports	No reports	These agents showed strong protoscolecidal effects (100% killing) at different concentrations and incubation times	Lack of reports of clinical application in surgery and PAIR	Further experimental testing and clinical trials strongly advocated to assess their practical application	
Other agents	Warm water (144), honey (173), propolis (174)	No reports	No reports	These agents showed strong protoscolecidal effects (100% killing) at different concentrations and incubation times	Lack of reports of clinical application in surgery and PAIR	Further experimental testing and clinical trials strongly advocated to assess their practical application	
Physical protoscolecidals	nsPEF (175), RFA (176)	RFA tested in human and animal surgery	Not assessed	nsPEF exhibited a good protoscolecidal effect	Low availability and high cost	Further assessment strongly recommended	

[a]Abbreviations: PAIR, puncture, aspiration, injection, and reaspiration of the cyst content; FBG, fluoride-containing bioactive glass; nsPEF, nanosecond pulsed electric field; RFA, radiofrequency thermal ablation.

Anti-infective treatment. Systemic anti-infective treatment relies on continuous administration of 2 benzimidazole carbamates, ABZ and mebendazole, which are the only anti-infective drugs clinically efficient to interrupt larval growth of *Echinococcus* spp. (177, 178). Mebendazole was the first benzimidazole that was proven efficient for the treatment of echinococcosis (179, 180). Because of its increased bioavailability and easier administration to patients, ABZ was then preferred as the anti-infective treatment of choice for echinococcosis, at an average dosage of 15 mg/kg/day (181). Currently, mebendazole is only an alternative drug for those patients who have experienced severe hepatic adverse effects with ABZ. Most of these patients experience similar adverse effects with both drugs; however, some individuals may tolerate mebendazole, which is critical when the patients cannot be operated on and their survival totally depends on the anti-infective treatment, a situation more frequent in AE than in CE (182). In CE, anti-infective treatment alone is reserved for small or medium-sized isolated cysts or, alternatively, multiple and inoperable cysts in the liver and/or in multiple organs. A combination of interventional techniques with ABZ is recommended and is used routinely with PAIR and derived techniques; it is less widely used with surgery (86, 89, 183, 184). However, criteria for curtailing anti-infective treatment are clearly missing and deserve prospective studies to be undertaken, and treatment length and schedule are still a matter of debate; a prospective study on a limited number of patients showed that 3 months of AE treatment was no better than 1 month of administration after PAIR (63). Based on pharmacological evidence and the relatively low and slow efficacy of ABZ to kill protoscoleces, a reasonable compromise would be to administer ABZ from 1 week before to 2 months after the interventional procedure (surgery or PAIR) whenever the cyst has been opened; however, firm recommendations should await the results of real studies, especially for the association with surgery. The "watch-and-wait" strategy is recommended for asymptomatic and small CE1 cysts, obviously degenerating CE4 cysts, and all CE5 type cysts (89).

A more systematic use of total cystectomy (also known as periadventitial cystectomy or, incorrectly "pericystectomy"), modified by the Chinese surgeon Peng Xinyu (Fig. 8) (89), has increased over the past 15 years (185, 186), and the attitude of surgical teams regarding total cystectomy is currently changing. To prevent recurrence, total cystectomy, which avoids cyst opening, is the technique of choice (Fig. 7). When the cyst is adjacent to major vessels, sub-total cystectomy, which avoids dissection of these vessels, is encouraged. If both techniques are not feasible, hydatidectomy (also called "endocystectomy" or "partial cystectomy"), after cyst opening, may be used together with obsessional prevention of protoscolex spillage, the major cause of recurrence, during surgery and with perioperative ABZ administration.

Since the first report of perlaparoscopic treatment of a CE patient in 1992 (187), robotically assisted (188) and single-incision laparoscopic total cystectomy and hepatic resection (189) have put laparoscopy into CE surgical practice (190). When laparoscopic surgery is performed, there must be no compromise to the principle of avoiding cyst content spillage and respecting cyst wall integrity; however, the influence of the laparoscopic approach on recurrence is controversial (191). For those CE patients with obvious biliary communication and unsuited for total cystectomy, "double drainage" of the fistula and cystic duct (i.e., drainage of the main bile duct with a Kehr T-tube through the cystic bile duct and drainage of the fistula or of the remaining cavity after partial cystectomy) is now preferred to reduce postoperative biliary leakage. In case of postoperative biliary leakage, a perendoscopic drainage (after endoscopic retrograde cholangiopancreatography [ERCP]) should be considered before any reoperation (192, 193). Liver transplantation for CE could be the last option in selected cases (194).

PAIR has definitely become part of the interventional therapeutic options in CE for midsized CE1 and CE3a cysts (63) (Fig. 7). A recent improvement in PAIR is the "modified catheterization technique" (MoCAT), a procedure appropriate for cysts up to 10 cm in diameter that includes aspiration of the parasitic membranes in addition to the cyst content and with a catheter left in place for the postintervention period of time. (195). When used by experienced operators, this technique may be an alternative to surgery

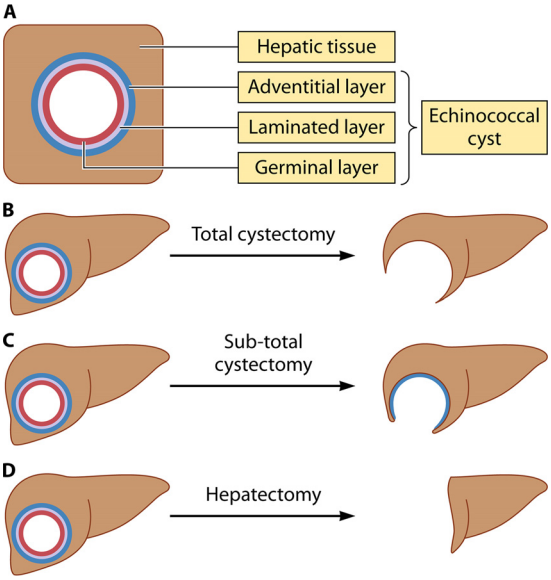

FIG 8 Schematic structure of the echinococcal cyst and different approaches for surgical removal. (A) The echinococcal cyst is made up of the adventitial layer, laminated layer, and germinal layer (from outside to inside). (B) Total cystectomy involves resection of the entire adventitial layer ("subadventitial resection"), the laminated layer, and the germinal layer. (C) Sub-total cystectomy involves partial resection of the adventitial layer and total resection of the laminated layer and the germinal layer, leaving parts of the adventitial layer in place whenever the operation is difficult because of the proximity of large vessels and/or adhesions. (D) Hepatectomy involves the *en bloc* resection of the echinococcal cyst along with part of the normal liver parenchyma. Partial cystectomy, which requires opening of the cyst, may leave all or part of the laminated layer and germinal layer and relies on the efficacy of a protoscolecide to destroy the metacestode; it should generally not be considered because of the potential for recurrence.

for noncomplicated CE2 and CE3b cysts (Fig. 5 and 7). Recent reviews have confirmed the efficacy and safety of PAIR and its variants if a stage-related strategy and technical recommendations are strictly followed (86, 89, 185).

Treatment of AE

With AE, therapeutic decision is based on the possibility of complete resection of liver lesions, after multidisciplinary assessment involving liver imaging, the general status of the patient, and the technical capabilities of the surgical team (86, 178, 182). As AE lesions are most often located in the right liver lobe and in advanced cases have invaded the major bile ducts and vessels (portal veins, hepatic veins, and vena cava), major hepatic surgery is often required, with significant morbidity and mortality resulting because of uncontrolled bleeding or liver failure. A number of cases cannot be safely operated on, even by highly experienced hepatic surgeons; only left hepatectomy in the less-frequent cases, where lesions are located in the left lobe, is accessible to less-experienced, nonspecialized surgeons. This explains why only one-third of patients with AE may benefit from curative liver resection, and the number is even lower in communities where AE is endemic and patients live in remote places and are late in seeking care (63). Palliative operations have been shown to be a source of complications without improving patient survival; it is the reason why they are now not recommended (63, 86). Objectives for the treatment of AE thus include the following: (i) totally removing the parasitic lesion, which is achieved through "curative" surgery combined with 2 years of ABZ treatment at the same dosage and with the same precautions as for CE treatment; (ii) if this is not possible, reducing the proliferating potential of the *E. multilocularis* metacestode by continuous administration of ABZ; and (iii) alleviating complications, especially bile duct obstruction and cholangitis and bacterial infection of the necrotic cavity that develops in the centers of advanced lesions (Fig. 6). Lesions which are massively calcified and/or negative by FDG-PET may

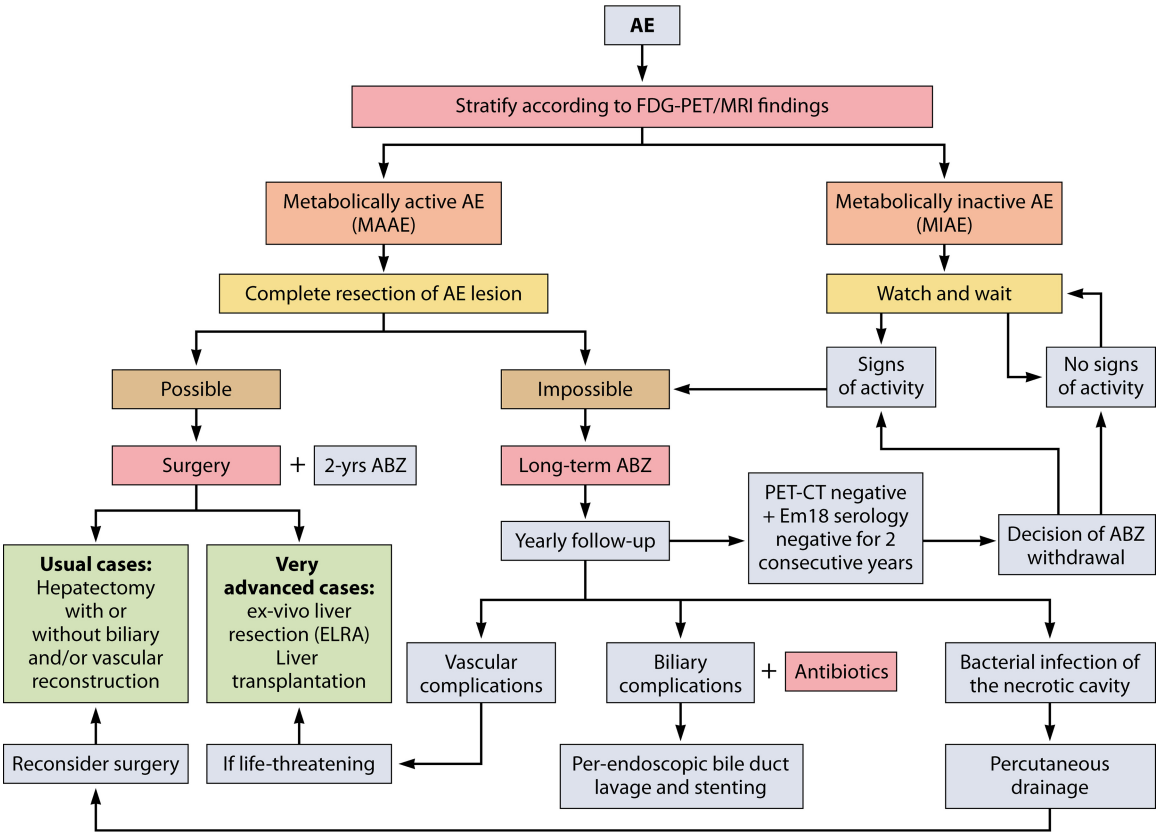

FIG 9 Algorithm for the treatment of alveolar echinococcosis. FDG-PET, fluorodeoxyglucose-positron emission tomography (increased uptake of FDG by the periparasitic immune response is the currently accepted evidence for AE lesion metabolic activity) (94). MRI, magnetic resonance imaging (identification of typical microcysts on T2-weighted images at MRI is a surrogate marker for AE lesion metabolic activity) (98). ABZ, albendazole; ELRA, *ex vivo* liver resection with autotransplantation.

benefit from a "watch-and-wait" approach. An algorithm that describes the recommended therapeutic strategy for AE is given in Fig. 9.

In immunocompromised patients, reducing immunosuppressive treatment must be considered when this is possible. In the French cohort, ABZ efficacy was shown to be fast and excellent but with more adverse effects than in nonimmunocompromised patients treated in the same centers. Whenever possible, and depending on the prognosis of the associated disease, curative liver resection should be performed as early as is realistic because of the fast growth of the metacestode in this situation and in order to facilitate the care management of the associated disease. Liver allotransplantation (77) is still used in advanced cases, especially when hepatic veins and the vena cava are included in the parasitic lesions, in case life-threatening complications result, but the shortage of donors and life-long immunosuppressant administration, which is followed by higher susceptibility to disease recurrence, have discouraged application of this approach (182). The high rate of postoperative morbidity and mortality (30% within the first 6 months after transplantation), as well as the recurrence rate (10% locally and 20% for distant metastases), in a recent report from Turkey even raises an ethical question, especially when the livers are from living donors (196). *Ex vivo* liver resection followed by autotransplantation is a surgical procedure for excising lesions following removal of the liver from the patient; the remaining lesion-free liver is then reinserted, similar to a liver transplantation (197) (Fig. 10). The procedure was initially developed to treat conventionally "unresectable" tumors as it does not require an organ donor and postoperative immunosuppressive treatment (198, 199); it was first applied to patients with advanced AE in 2011 (197, 200, 201). AE patients often present with hypertrophy of the liver lobe not invaded by the parasitic lesion (because of

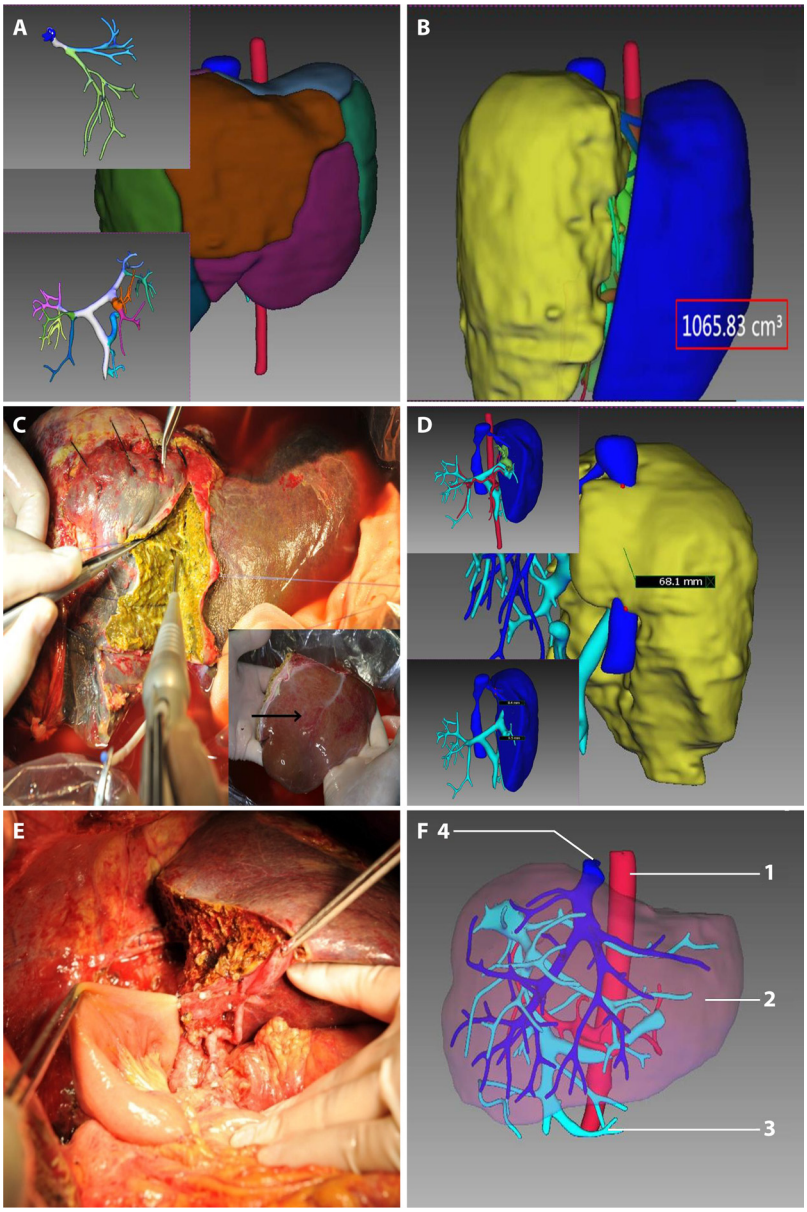

FIG 10 Three-dimensional reconstruction in the application of *ex vivo* liver resection and autotransplantation (ELRA). (A) Portal veins, hepatic veins, and segments of liver are visualized by three-dimensional reconstruction. (B) Calculation of remnant hepatic parenchymal volume after three-dimensional reconstruction and virtual resection. The volume of remnant liver is 1,065.83 cm^3 (yellow, giant AE lesion; blue, normal parenchyma). (C) Precise resection of giant AE lesion on bench (black arrow, normal liver parenchyma after resection). (D) The length (68.1 mm) of obliterated retro-hepatic vena cava is calculated through three-dimensional reconstruction (yellow, giant AE lesion; blue, retro-hepatic vena cava). (E) Hepaticojejunostomy is performed on the table (black arrow, anastomosis). (F) Postoperative follow-up demonstrating liver remnant and vasculatures (1, aorta and hepatic artery; 2, liver; 3, portal vein; 4, hepatic vein).

chronic long-term portal vein obstruction and/or a specific influence of the immune response to the parasite which favors hepatic regeneration). This is one of the reasons for a relatively favorable outcome of the procedure (and of major hepatectomies in general) compared with cancers which develop rapidly and do not promote hypertrophy of the remaining liver lobe (202) (Fig. 10). Midterm results of such operations seem acceptable in comparison to conventional major hepatectomy with complex bile duct and vessel reconstruction or to liver transplantation (203, 204). With an average follow-up of 22.5 months (range, 14 to 89 months) in 69 patients, overall mortality was

12%, complications higher than IIIa (according to the Clavien classification) were observed in 10 patients, and there was no recurrence (204). However, long-term results and comparison with nonsurgical care management, including long-term ABZ and perendoscopic treatment of biliary complications, are not yet available.

European data indicate a marked trend toward a reduction in the percentage of surgical operations for AE, whatever their type (complete or only partial resection of the lesions, or any surgical procedure such as bile duct derivation, or simple diagnostic laparotomy) but an increase in the percentage of radical/curative operations, i.e., liver resections capable of totally removing the metacestode tissue from the liver and/or other organs (178, 205, 206). However, the percentage of patients undergoing surgery has remained high in China (207). The differences are mainly due to recruitment of patients (either symptomatic with advanced lesions, most often in China, or asymptomatic with less-developed lesions, most often in Europe) and also, whatever the continent, to the specialization, experience, and boldness of the surgical teams and appreciation of the "safety margin" necessary to perform an R0 resection (according to the grading followed in cancer surgery). Evaluation of the influence of this safety margin suggests that at least a 1-mm distance is important (thus, being less than in cancer) combined with ABZ therapy (208). Difficulties in the strict follow-up of patients, because of their residence in remote areas, in measurement of ABZ sulfoxide (ABZ-SO) to monitor adherence to treatment (which is crucial for the success of an anti-infective drug approach) and the origin of adverse effects, and differences in the organization of health care management, may also be among the reasons that make Chinese teams more prone to favor a surgical approach.

Percutaneous puncture for treating AE patients with a necrotic cavity inside liver lesions and bacterial superinfection has been used for more than 30 years, and, in combination with antibiotics, the procedure may save patients and allows a reassessment of the resectability of the lesion (83, 178, 192). Injection of protoscolecidal agents should never be used in AE: in fact, the central cavity often observed in advanced cases of AE is due to the necrosis of the lesions, including the multiple degenerating microcysts of the metacestode and associated immune infiltrate and fibrosis; the still active microcysts are at the periphery of this cavity, as is well shown by FDG-PET images and T2-weighted MRI (98). These microcysts are embedded in the immune and fibrotic reaction and are not accessible to a protoscolecidal agent; in addition, at this stage, communication of this central cavity with bile ducts is the rule, and such an injection would be not only useless but also harmful (178). Early and/or late biliary complications clearly heavily impact the immediate prognosis of the disease, at presentation and within the first year of follow-up, and thus the final outcome of AE (85, 209); they represent a negative turning point in the course of the disease (210). Percutaneous dilation of the bile ducts obstructed by the progression of the metacestode was widely performed until the end of the 20th century, instead of palliative surgical bile diversion (83, 209, 210).

A European survey of perendoscopic procedures (through ERCP) to treat biliary complications of AE in 18 clinical centers showed that such procedures are now used routinely and are generally successful in alleviating symptoms and in maintaining long-term permeability of the biliary strictures; to achieve good results, extensive saline lavage of the bile ducts, which removes necrotic debris and intrahepatic biliary stones that are common in such patients, and the use of multiple plastic stents are recommended (211) (Fig. 6). Perendoscopic bile duct stenting is currently nearly totally replacing surgical palliative operations and percutaneous biliary drainage to treat biliary complications in AE patients (Fig. 6). Although there have been no specific studies assessing the quality of life of patients receiving such treatment, we may anticipate that this has markedly contributed to the improved quality of life of those patients with chronic biliary obstruction and multiple cholangitis episodes; in the past, more than 10 reoperations in a single patient were not uncommon, and most patients had a very uncomfortable external biliary drainage tube for life (211).

In Europe, all retrospective evaluations of survival after AE diagnosis have indicated that there have been major improvements in the 21st century compared with the prior 30-year experience; improvement was already noted in the 1990s compared with the 1970s, being mostly due to earlier diagnosis, the introduction of anti-infective treatment, and the progressive abandonment of palliative surgery (85, 212). The situation remains worrisome, however, in countries/regions with limited medical facilities and where diagnosis is performed only at an advanced stage of disease, as well as for those patients who experience severe side effects of benzimidazoles, as there is no alternative. Until now, the numerous attempts at finding new drugs or at converting drugs already used in other parasitic diseases for their application to the echinococcoses have essentially failed (213). Drugs that showed some promise *in vitro* and in experimental animals, and were effectively tested in humans or domestic animals, include other benzimidazole compounds such as flubendazole and oxfendazole, as well as nitazoxanide (182). Lists of those drugs that were tested *in vitro* and found not suitable for use in humans after being tested *in vivo* in experimental animals are available in more-specialized reviews (182, 213). There is some hope from *in vivo* experiments with mefloquine (214–218) and artemisinin (219, 220) derivatives, and very recently, derivatives of carbazole aminoalcohols have been shown effective against cysts of *E. granulosus* both *in vitro* and *in vivo* (221). However, none of these compounds have, as yet, been subjected to pilot clinical trials.

CE and AE Disease Follow-Up

Long-term imaging follow-up after initiation of treatment (more than 10 years) has long been stressed for AE (86, 222). It is now accepted that a close follow-up of patients with CE should also be undertaken for at least 5 years because of the high rates of relapse after surgery and the uncertainty of complete cure after drug treatment and/or percutaneous puncture. Regular tests of blood counts and serum transaminases are necessary to assess the safety of management within the first 6 months after initiation of anti-infective treatment, since hepatic toxicity and leukopenia are the most severe adverse effects and may prevent ABZ use in some patients. ABZ-SO or mebendazole measurements are extremely useful to assess the patient's observance and to adjust drug dosages. ELISAs using HF antigens and/or purified AgB and Ag5 for CE and EM2-plus or Em18 ELISA, if available, for AE exhibit high performance in detecting disease recurrence after surgical resection of the cyst/lesion, although they are less accurate if all or part of the cyst/lesion remains in the infected organ (182, 223). FDG-PET is currently considered the "gold standard" for the evaluation of the metabolic activity of AE lesions and for decisions about anti-infective treatment interruption; however, its predictive value is far from perfect, despite technical improvements (e.g., delayed image acquisition 3 h after FDG injection [224, 225]), and all other imaging techniques deserve more evaluation (97).

There has been recent active searching for parasite viability/disease progression markers in the sera of AE patients (226), with antibodies against recEm18 showing promise for long-term monitoring (227–229); however, when used alone, the test is not sufficiently discriminant to make a decision on treatment withdrawal. Double-negative results for FDG-PET and anti-recEm18 antibodies currently represent the best marker to consider treatment interruption (228). In all patients, recommended follow-up is at 1, 4, and 12 weeks for the first 3 months after diagnosis and initiation of ABZ, to check for ABZ adverse effects by measuring blood cell count and transaminases, and whenever possible, adjusting the treatment based on ABZ-SO measurement. The patient then is asked to present every 3 months for the first year and every 6 months until the end of the second year; this follow-up includes US, blood count, transaminases, and serology, and ABZ-SO is also measured 2 to 4 weeks after each dose adjustment, if necessary (182). In all patients FDG-PET is performed at the end of the second year. In patients with curative surgery, ABZ treatment is withdrawn 2 years after surgery if there is no recurrence as assessed by US, FDG-PET, and serology; yearly follow-up using US and serology is then recommended until 10 years after surgery. In patients without curative

surgery, after the second year, yearly follow-up includes US, blood count, transaminases, and gamma-glutamyl transferase measurement, and serology, with FDG-PET-CT being performed every 2 years. A decision on anti-infective treatment withdrawal is made after at least 2 consecutive negative FDG-PET and Em18 serology assessments.

Detection of circulating serum or plasma *Echinococcus* sp. antigens (CAg) may be an alternative approach to serology. Early studies showed that *E. granulosus sensu lato*-specific circulating antigens, positive in 75% sera of antibody-negative CE patients, were associated with the growth dynamics and activity of cysts (230). Sensitivity of CAg detection, however, varied between 21% and 85%, mostly owing to the formation of circulating immune complexes. To our knowledge, only preliminary studies have been performed (231) to determine whether antigen detection may be a useful approach for assessing the efficacy of treatment, especially after removal of the cyst, and no studies using circulating antigens for long-term disease follow-up on a significant number of patients with CE or AE are available (36).

Assessing circulating cell-free DNA (cfDNA) could also be an option, as its detection as a biomarker has proved useful in cancer, and it has shown some promise in parasite diagnosis; for example, infections with all the three major human schistosomes (*Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum*) have been identified with PCR-based cfDNA assays using both species- and genus-specific target genes in animal models and patients (232). However, with the currently available detection methods, the approach does not seem sensitive enough for use in clinical practice, at least for AE, due to the low levels of cfDNA detectable in patient serum (233).

PREVENTION AND CONTROL

Current prevention and control of CE relies on the provision of safe animal slaughtering conditions (offal destruction and preventing dogs from feeding on infected organs of ungulates) and on dosing dogs with praziquantel (234). With notable exceptions, such as New Zealand, Tasmania, Iceland, Cyprus (at least temporarily), Chile, and some provinces in Argentina (3), other attempts at control have been generally disappointing. In countries where very strict slaughtering measures have been implemented, leading to the near disappearance of human CE cases, such as mainland Australia, persistence of a wild cycle of *E. granulosus sensu stricto* makes a reappearance of the disease always possible (39). Evaluation of control programs shows that (i) success is more readily achieved on islands, (ii) a combined multidisciplinary and multi-institution, and often multicountry, effort is necessary, and (iii) a One-Health approach is required (235, 236). An ambitious, well-financed, and state-driven control program, including community US screening, care management of diagnosed patients, and monthly dog dosing with praziquantel, is in operation across western China. The monthly dosing of dogs is suitable for village-based communities (237) but is far less effective in seminomadic or pastoral areas (238).

Vaccination of sheep with the EG95 vaccine has been promoted as a complementary intervention to eliminate CE transmission (14), and there have been trials of this approach in China and South America. For most countries where CE is endemic, however, the logistics and costs of vaccinating sufficient numbers of animals may preclude widespread application of the vaccine. Dog vaccination would be an effective complementary intervention for controlling echinococcosis transmission (239), although recent progress has been slow (240). Nevertheless, development of a single vaccine, effective against both *E. granulosus* and *E. multilocularis* in canines, may be feasible and would be practical given that the two species are sympatric in many countries of the Northern Hemisphere. Besides the red fox, the main definitive host of *E. multilocularis* (241–243), other carnivores such as the raccoon dog and domestic dog also act as definitive hosts (32, 244, 245). The role of dogs in AE transmission is especially important in western China and in central Asia (53, 246) and may be more relevant in Europe than previously considered; conversely, a wildlife cycle may also be of concern for CE, especially in Africa (13, 247). Baits impregnated with praziquantel

have been applied against *E. multilocularis* (248, 249), and a bait-delivered vaccine, when available, could be used to interrupt the parasite's transmission cycle in wildlife, notably foxes, in cities and parks (250) and in selected rural areas (31, 251).

A successful control campaign should focus on the most at-risk areas and on those animal hosts mainly involved in transmission, with progress constantly monitored (10, 17, 252, 253). Close surveillance of the prevalence of *Echinococcus* spp. in dogs/foxes is extremely important for evaluating the progress of a control program (254). Detecting and quantifying *Echinococcus* sp. eggs/proglottids in canine fecal samples is recommended as an alternative to necropsy. Major improvement in parasite DNA detection in feces and environmental samples has occurred in the last 15 years, and this is currently preferred to antigen-based diagnosis (126, 140, 255–259). As indicated above, LAMP-based assays are useful as a first-line screen for *Echinococcus* spp. in the field (134–137), and PCR methods allow combined identification of definitive host species and *Echinococcus* sp. infection status using feces collected in the field (260). Adaptation of the available molecular methods to detect *Echinococcus* sp. egg DNA in environmental samples (e.g., in soil, water, and sewage and on vegetables) is an important step to better identify high-risk areas and the actual routes of human infection (138–140), leading to more effective control.

RECENT APPLICATIONS OF OMICS TECHNOLOGIES

Improving Understanding of the Complexity of *Echinococcus* Species Life Cycles and Unravelling Species-Specific Phenotypic Differences

Gene transcript analysis of representative CE life stages (protoscoleces, cyst germinal cells and membranes, adult worms, and oncospheres) has allowed exploration of different aspects of tapeworm biology and parasitism (261). Further, the recent publication of the complete genomes of *E. granulosus* (261) and *E. multilocularis* (262) has revealed other key features associated with parasitism, including a description of a number of domain families gained during the course of evolution. Other important genes identified include those associated with strobilization and reproduction, signaling pathways, and neuroendocrine and nervous systems and others involved in evasion of immune recognition and regulation of host immunological responses. The genome and transcriptome data therefore provide a critical basis for more-detailed understanding of cestode biology, differentiation, development, evolution, and pathogenesis and other host-parasite interactions.

The complex life cycles of *E. granulosus sensu lato* and *E. multilocularis* provide a platform for addressing the functions of the expressed products of novel genes. Up- or downregulation of gene expression likely underpins the phenotypic changes associated with the different life cycle stages and the respective modulation of the immune response that each species determines. In-depth transcription analysis is critical for searching for key genes associated with these changes and for identification of their specific functions. One of the unique characteristic physiological features of *E. granulosus sensu lato* and *E. multilocularis* is the remarkable ability of the protoscolex to differentiate into an adult worm or to dedifferentiate into a cystic stage. Specific host stimuli (bile acids) govern the direction of development (263–265), and relevant parasite-expressed receptors and transporters likely stimulate the relevant developmental pathways. Gene function prediction analysis was unable to attribute a function to 3,900 of the 11,325 genes predicted to be present in *E. granulosus sensu stricto*; among these, 361 genes were transcribed in adult worms, of which 21 were highly expressed and may be associated with adult worm development (261). The morphology of the adult worms of *Echinococcus* spp., though following the typical taeniid cestode pattern, has only up to 5 immature, mature (with reproductive organs), and gravid proglottids sequentially present, which are replicated through strobilization. The gravid proglottid contains eggs which are released into the environment to infect intermediate and human hosts. Of the 361 genes shown by mRNA transcript analysis to be highly expressed in adult *E. granulosus*, 55 were specifically expressed compared with the oncosphere and cyst stages, in which these genes are silenced. The metaces-

tode stage is associated with unlimited asexual development, whereas adult worms have limited, sexual development; of 8,361 genes expressed in the two stages, 498 and 502 genes were highly expressed in the adult worm and in the metacestode, respectively (261). Future work aimed at posttranscriptional suppression of these genes through RNA interference (RNAi) and gene knockout techniques may unravel their functional characteristics.

A comprehensive comparison of the genomes and transcriptomes of *E. granulosus* and *E. multilocularis* will also be central to our understanding of the biological/pathological differences between the two species. A major difference between the two is the morphology of the metacestode. *E. granulosus* has a unique cyst formation, with a shell-like adventitia which clearly separates the cyst from the surrounding (liver, lung, and brain) parenchyma. Conversely, the *E. multilocularis* metacestode is an infiltrating lesion composed of aggregated microvesicles, cells of the intermediate host's immune response, fibrosis and necrosis, with no clear edge to the lesion, which continuously progresses eccentrically and damages the liver or other target organs. Comparative analysis of divergent and convergent gene pairs and their pattern of expression using microarray technology or RNA sequencing (RNA-Seq) is a relatively recent approach that can be used to identify patterns that are shared by more than one species or are unique to a particular species, with the capacity to reveal differences in biological phenotype. Although in its infancy for studying *Echinococcus* spp., such gene pair analysis has revealed that *E. granulosus* and *E. multilocularis* have 10,018 genes with high sequence similarity, with 5,418 being identical. The next stage will be to identify and characterize nonsimilar/unique genes so as to shed light on the inherent differences in morphology or pathology between the two tapeworms.

Improving Diagnosis and Drug Treatment of Echinococcosis

The currently available rich genomic and transcriptomic data may be useful for developing new public health interventions against echinococcosis; these include improved diagnostic tests and the identification of new drug targets. BLAST sequence analysis of the *E. granulosus sensu stricto* genome indicated that one-third ($n = 3,903$) of the genes present have no gene homologues or orthologues in other taxa, suggesting that these genes are probably *Echinococcus* specific, likely underpinning the unique features and biological characteristics of *E. granulosus sensu lato*. The products of these genes may also be of value as new candidates for diagnosis and as novel drug targets for the treatment and control of echinococcosis. Some proteins likely serve as messengers for cross talk between *E. granulosus* and its hosts and may prove useful as chemotherapeutic targets as well as for improved immunodiagnosis or immunotherapy (261, 262). Potentially "druggable" proteins (i.e., polypeptides which might be the targets of new or existing drugs) expressed by genes in the germinal layer of the metacestode include G-protein-coupled receptors (GPCRs), serine proteases, ion channels, and neuropeptides (266) and components of the mitogen-activated protein kinase (MAPK) pathway (267–270).

Hormone- and cytokine-activated pathways have been identified in both *E. granulosus sensu stricto* and *E. multilocularis* metacestodes (271–273), and their activation/inactivation by host components is highly suggested (267, 269, 270, 273–281). Importantly, comparison of the *E. granulosus* and *E. multilocularis* genomes indicates a high level of gene sequence similarity, suggesting that the two parasites may share many common molecules that can be targeted for developing new interventions. MAPK inhibitors are currently being actively studied for their killing effects on the metacestode and/or protoscoleces. An ATP-competitive pyridinyl imidazole inhibitor (ML3403), targeting the P38-like MAPK from *E. granulosus sensu stricto,* effectively suppressed Egp38 activity, which led to significant protoscolex death within 5 days *in vitro* (267). Similar results were obtained with *E. multilocularis*; ML3403, in particular, and SB202190, another pyridinyl imidazole, tested on metacestode vesicles cultured *in vitro* led to dephosphorylation of the parasite's EmMPK2 and subsequent killing of the parasite vesicles at concentrations that did not affect cultivated mammalian cells (270).

As a direct result of the unveiling of the complete genomes of the *Echinococcus* spp., several metabolic pathways have been explored, and other inhibitors are currently being studied (177, 282–287). Nilotinib, an ABL (Abelson murine leukemia viral oncogene homologue)-tyrosine kinase inhibitor, and everolimus, a serine/threonine kinase inhibitor, caused alterations of *E. multilocularis* metacestode vesicles *in vitro*; however, neither of these compounds resulted in any reduction of parasite growth in *E. multilocularis*-infected mice, and combined application of the kinase inhibitors with ABZ did not lead to synergistic or additive treatment efficacy (282). BI2536, a Polo-like kinase (a kinase containing Polo box domains) inhibitor that has been tested in clinical trials against cancer, was shown to inhibit EmPlk1 activity and to block the formation of metacestode vesicles from cultivated *E. multilocularis* germinal cells; furthermore, it eliminated the germinal cell population from mature metacestode vesicles *in vitro*, yielding parasite tissue that was no longer capable of proliferation (286). Similarly, imatinib, another ABL tyrosine kinase inhibitor used to treat cancer, has been shown to interact with the ABL-like kinases present in *E. multilocularis* and to be highly effective in killing *Echinococcus* stem cells, metacestode vesicles, and protoscoleces *in vitro* (287). However, the potential of these kinase inhibitors to treat AE *in vivo* is as yet unknown.

Improving Understanding of Immunological Mechanisms of Host-Parasite Interactions To Develop Immunotherapy

Although the extreme susceptibility of *Echinococcus* spp., and especially of *E. multilocularis*, to the cellular immune response of the host has been well recognized since the 1980s (288), much of the comprehensive knowledge of the immunological mechanisms at work in the subtle balance between host protection and parasite growth has been gained in the 21st century (76, 289). In this field, advances in genomics have been of help by suggesting additional molecular mechanisms/pathways and new therapeutic targets. In particular, studies of the transcriptional profiles observed in the livers of *E. multilocularis*-infected mice and the use of mouse models with specific gene deletions have been crucial (290–293). Recently obtained information suggests strongly that immunotherapy could complement the anti-infective drug approach to treat echinococcosis. Conversely, a better understanding of the immunological profiles of intermediate hosts infected with *Echinococcus* spp. may add new tools to the therapeutic arsenal targeting chronic inflammatory diseases.

The predominance of a T helper 2 (Th2) profile, including interleukin-5 (IL-5)- and IgE-dependent reactions, and high levels of IL-10 cytokine at the chronic stage of both AE and CE in humans have been known for some time (64). In CE, a dominant Th2/T regulatory (Treg) cytokine profile is rapidly established after the formation of the adventitial fibrous barrier (293, 294). In AE, the immune response follows a 3-stage course characterized by a mixed Th1/Th2 profile at the early stage, a dominant Th2/Treg profile, including IL-10 and transforming growth factor β (TGF-β) regulatory cytokines, at the chronic middle stage, and a T-cell exhaustion status at the final stage of infection (289, 295). Clinical studies on CE have shown the response to anti-infective therapy (or of "inactive" cyst) is associated with a Th1 profile, whereas on the other hand, resistance to treatment (or of "active" cyst) is associated with a Th2 profile and elevated IL-10 levels (289, 293). Clinical studies in AE suggest that a combination of Th2-related cytokine serum levels, such as those of IL-23 and IL-5, could be used as a surrogate marker of AE metabolic activity in humans (294). Some proteins likely serve as messengers for cross talk between *E. granulosus* and its hosts and may also prove useful as targets for improved immunodiagnosis or patient follow-up (261, 262).

The composition and type of the periparasitic immune response elicited by *Echinococcus* sp. infection causatively influence the outcome and progression of disease, ranging from resistance (self-cure) to rapidly evolving host fatality (high susceptibility) (291). In *E. multilocularis*, the parasite load, which can be quantitatively assessed in an experimental model involving infection by intraportal injection of protoscoleces, significantly influences this periparasitic response as well as the systemic cell and cytokine

profile (295). Recent experimental studies show that Th1/Th17 polarization is a pivotal factor for resistance, while FoxP3$^+$ Tregs are key players in the immune regulatory processes favoring *E. multilocularis* metacestode survival (296). *In vivo* treatment of mice by a single intravenous injection of 200 μl recombinant IL-17A at the optimal concentration of 125 pg/ml 2 weeks after *E. granulosus sensu stricto* infection decreased the infectivity rate by 2/3 and reduced metacestode growth by more than 90% (297). FoxP3 Treg depletion after infection using the DEREG (depletion of regulatory T cell) model in mice (296) and also genetic inhibition of the synthesis of fibrinogen-like protein 2 (FGL-2), a CD4$^+$ CD25$^+$ Treg effector molecule (298), are able to control *E. multilocularis* secondary infection.

In the per-oral model of infection, which better mimics AE in humans, the potential of FoxpP3 as a good target for application in immunotherapy has been confirmed (299). Another promising candidate is the programmed death-1 (PD-1)/PD-ligand 1 (PD-L1) signaling pathway, which plays a critical role in the induction of Foxp3$^+$ CD25$^+$ CD4$^+$ Tregs, positively influences IL-10 and TGF-β secretion, inhibits effector T-cell proliferation and activation, and prevents Th1 cytokine production (300). Elevated soluble PD-L1 levels (301) and increased number of PD-1-expressing follicular helper T cells were observed in patients with CE compared with healthy controls (302); in experimental AE, percentages of PD-1$^+$ Tregs and PD-L1$^+$ dendritic cells (DCs) increased significantly together with levels of Foxp3, IL-10, and TGF-β during the chronic middle stage of infection (303). Preliminary experiments with a PD-1/PD-L1 engagement blockade are promising (304). Several PD-1/PD-L1 inhibitors already used in cancer treatment (305) are available to clinicians for pilot immunotherapeutic trials in AE. Combined anti-infective and immune therapy could also help clinicians in the management of severe, multiorgan cases of CE.

The specific immunological profile of the chronic stage of *Echinococcus* spp. infections has attracted attention as an established tolerance state that could be used to alleviate deleterious effects of inflammatory reactions in a variety of clinical conditions. Concomitant *E. multilocularis* infection in the rat delays rejection of a liver allograft (306), whereas concomitant *E. granulosus sensu lato* infection also reduces ovalbumin-induced airway inflammation of mice (307); in both situations, the effects were associated with raised IL-10 levels in the experimental animals. Although the potential efficacy of *Echinococcus* sp. components to treat rheumatoid arthritis was also evoked, it does not seem to have been demonstrated yet (308). The strongest evidence for the immunoregulatory role of established *Echinococcus* sp. infection in its murine intermediate host on an inflammatory disease has come from observations in experimental colitis. Both *E. granulosus sensu stricto* infection (309) and *E. multilocularis* infection (310) are able to reduce the development of dextran sulfate sodium (DSS)-induced colitis in mice. The possible use of noninfective *Echinococcus* sp. extracts is supported by observations made after treating mice daily, starting 3 days before colitis induction, with extracts from *E. granulosus sensu stricto* laminated layer; the treatment significantly improved the clinical symptoms and intestinal histological scores and maintained mucus production by goblet cells, while causing a significant decrease in gamma interferon (IFN-γ) and TNF-α and an increase in IL-10 production (311).

The immunomodulatory properties of *Echinococcus* sp. laminated layer have been well studied by reference to the shift they may induce in the immune response of the host to enhance metacestode growth and thus reduce host protection (76, 312). A variety of immunomodulating molecules produced by *Echinococcus* spp. have been identified, including antigen B (AgB) subclasses, Eg2 heat shock protein (Hsp) 70, and EgTeg from *E. granulosus sensu lato* (313) and Em2 (G11), EmAP, and *E. multilocularis* activin-like (EmACT) from *E. multilocularis* (290); they should certainly be reconsidered with a positive view to obtain the best combination of immunomodulating components to be used in inflammatory bowel diseases and, more generally, in all clinical situations that require tolerance induction.

Improving Vaccine Development

Vaccination of intermediate hosts. Vaccination of intermediate hosts of *E. granulosus* with the EG95 antigen has resulted in remarkable protective efficacy in pilot and field trials and is currently being used in areas of endemicity in China and South America (17, 314–317). The *Echinococcus* oncosphere is the infective stage for humans and intermediate hosts. Products of other genes differentially expressed by this stage likely represent potential additional vaccine candidates given that the protein expressed by the oncosphere-specific *eg95* gene induces a high level of protection against egg challenge infection in sheep and cattle (317, 318). Gene transcript analysis revealed that *eg95* is highly expressed in oncospheres (261), and recent studies show that *eg95* comprises a family of 7 distinct genes. Gene transcript analysis also showed that 340 (out of 3,811) genes were highly up-regulated in oncospheres compared with those in the adult and cyst stages of *E. granulosus* (261). Of the oncosphere-expressed genes, 2% (74/3,811) encode secreted proteins which likely play a key role in the penetration of the hatched oncosphere through the mammalian intestinal wall and in subsequent oncospheral development.

Vaccination of definitive hosts. A dog vaccine effective against adult *Echinococcus* sp. infection would be highly desirable as an intervention in integrated echinococcosis control. Such a vaccine is not currently available. The protoscolex is the stage which develops into an adult worm in the canine intestine. Proteins of genes highly expressed in the protoscolex or in the adult may provide suitable vaccine candidates against adult worms in the definitive host. Products of a novel, highly expressed *egM* gene family (e*gM4*, *egM9*, and e*gM123*) in mature adult worms, which may be associated with adult worm maturation and/or egg development, showed encouraging protective efficacy against adult worm infection in vaccine trials where dogs were vaccinated and necropsied 45 days after challenge infection (319, 320). Adult *Echinococcus* worms are localized to the middle of the small intestines of their definitive hosts, where abundant nutrients, especially amino acids, are present together with high levels of trypsin and trypsin-related enzymes. The worms secrete serine protease inhibitors (serpins) which counteract the potentially lethal effects of these host intestinal proteases and thereby likely play a key protective role in preventing proteolytic enzyme attack, ensuring survival of *E. granulosus* within its canine hosts. These, along with molecular chaperones, neurotransmitter receptors and transporters, and other protease inhibitors, specifically expressed in adult worms, likely represent additional vaccine candidates that warrant future study (261, 285, 321–323).

CONCLUSIONS AND FUTURE PERSPECTIVES

Awareness of clinicians and medical researchers of the public health importance of echinococcosis, even in areas where it is not endemic, is crucial, and an improved knowledge of echinococcosis imaging is essential for diagnosis and a prerequisite for multidisciplinary decisions on treatment strategy. For diagnostic confirmation, standardization and quality control of the currently available serological tests, both for diagnosis and for disease monitoring, is more of a priority than an everlasting quest for the "perfect antigen"; an easier recourse to molecular identification of the parasites ensures a more rapid and reliable species diagnosis.

For the care management of both CE and AE, a new concept, akin to that considered for cancer patients, has emerged, and the issue of recurrence, and thus of prolonged patient follow-up, is now taken seriously in CE. New surgical techniques make the complete resection of CE cysts easier and complete resection of AE lesions possible even in very advanced cases. The currently available techniques of nonsurgical interventional treatments have improved the quality of life of patients. However, prospective studies with prolonged follow-up are still needed to base echinococcosis therapeutic strategy on evidence. In addition, more than 30 years after the first trials of mebendazole and ABZ, there is no available alternative to these two drugs as antiinfective therapy, a situation unique in the field of infectious diseases. Fortunately, new biological or immunological therapeutic targets may now be more easily identified

because of new proteomics information, the complete sequencing of the *E. granulosus* and *E. multilocularis* genomes, and increased understanding of the host-parasite interactions in both AE and CE.

Controlling the transmission of *Echinococcus* spp. continues to be a considerable obstacle, but precise identification of the infecting species/genotypes may help public health institutions better focus and optimize the effectiveness of control programs. Involvement of wild animals in the life cycle of all *Echinococcus* species makes disease control dependent on landscape and climate changes and is, consequently, more challenging now than hitherto. Important improvements in molecular biology-based tests to detect *Echinococcus* spp. in definitive hosts and in the environment, however, make control program monitoring potentially easier. One challenge in the control of AE and CE in coming years will be to define optimum targets for developing vaccines effective in definitive canine hosts to interrupt the chain of transmission to humans if echinococcosis elimination, slated for 2050 by WHO, is to be achieved.

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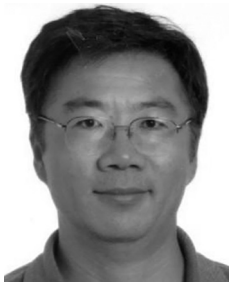

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